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THE INFLUENCE OF EXERCISE ON MUSCLE TONUS AS EXHIBITED BY THE KNEE-JERK

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From studies made upon himself, Lombard (1) reports that he always found the extent of the knee-jerk to be markedly decreased by a walk, or even by a short stroll. He gives the result of one experiment, the effect of walking up and down stairs for fifteen minutes, and states that muscular fatigue results in a decreased extent of the knee-jerk. Contrary to the observations of Lombard, Sternberg (2) found, while doing similar experiments, that fatigue increased the extent of the knee-jerk and concludes that an increase in its extent is an indication of fatigue. Ryan (3), after measuring the tension required to extend the pectoral or soleus gastrocnemius muscle groups, reports that strenuous work of short duration usually causes a decrease in the tonus of those muscles. He finds, further, that light work during a day following a good night's sleep frequently results in better tonus in the evening than was observed in the morning. In view of the contradictory results reported by those investigating this problem up to the present time, it seemed worth while to make a further study of this question, the results of which are herein reported.

Data were collected from thirty-four students in the School for Athletic Coaches by means of a specially constructed apparatus designed by Tuttle (4). This apparatus delivers blows of uniform intensity at the constant rate of seven per minute to the patellar tendon, and in this series of experiments a blow of constant strength and rate was used throughout. In using the apparatus, the subject is seated in a barber's chair which has been so altered that it can be readily adjusted to accommodate subjects of various sizes. The record is made by attaching a stirrup to the heel of the subject's shoe, running a string from this through a reducing pulley to a stylus which is suspended from a spring. The forward component of the excursion of the foot, as recorded by the stylus on a slowly moving kymograph, is used as the index of the knee-jerk and is referred to as

the "height" or "extent" of the knee-jerk. The stylus records a base-line, and a given excursion is measured in millimeters from the baseline to its end.

To make sure that the subjects would be at ease when the records were made, preliminary training was given in each case by the taking of a ten-minute practice record. The subjects were instructed to assume and maintain a comfortable position so as not to change the point of impingement of the stimulus. The subjects were also instructed to be as passive as possible and were left alone in the room while the records were being made. For this series of experiments each subject was placed in the chair and allowed to deliver normal kicks for twenty minutes, after which he carried out certain forms of exercise designated by the experimenter. The point of impingement of the stimulus used before exercise was carefully noted in order to eliminate the possibility of striking a different position when the record was taken after exercise. Immediately after exercise the subject was again placed in the chair and allowed to deliver kicks for twenty minutes.

In this series of experiments no attempt was made to standardize the exercise assigned to each subject. An attempt was made, however, to make the exercise sufficiently strenuous so that at least a part of the group would experience the feeling of fatigue. The group was selected more or less at random except that an attempt was made to secure some who were used to more strenuous exercise than others.

When they returned from their exercise, the subjects were not questioned as to the extent of their fatigue. The classifications shown in the columns marked "extent of fatigue," tables 1 and 2, were based on statements volunteered by the subjects and on their appearance and actions.

The writer is intimately acquainted with the subjects and their habits in exercise and was thus able to determine with a high degree of accuracy the physical fitness of the subjects. In general, those who had been in practice for a position on a varsity athletic team for some time and those who have a special pride in their good physical condition were considered to be above average. Those who are indifferent in their habits of exercise or who have certain handicaps were considered to be below average. The rest of the subjects were considered to be average in fitness. The writer does not intend for the terms used in describing the extent of fatigue or the degree of fitness to carry implications other than those set forth for use in this experiment.

Since a part of the subjects used in this experiment showed an increase in the height of their knee-jerk after exercise, and a part of them showed a decrease, the data are presented under two headings:

A: Subjects who showed an increase in the extent of their knee-jerk after exercise.

B: Subjects who showed a decrease in the extent of their knee-jerk after exercise.

For four out of the thirty-four subjects the blow used was subminimal both before and after exercise and, consequently, their records are not considered further.

A: Subjects who showed an increase in the extent of their knee-jerk after exercise. Fifteen subjects are included in this group, and the data obtained are shown in table 1.

The records of subject 30 are representative of this group and are shown in figures 1 and 2. The subject is 21 years old, a sophomore in the University, and has a pride in maintaining himself in fine physical condition, but is not on any varsity athletic squad.

For these fifteen subjects we find that, as a group, the height of their kicks after exercise was 1.51 times greater than before exercise. The data further show that six in the group were above average in degree of physical fitness, and that the height of kicks of these six was increased 1.80 times after exercise. Seven of the group were average in physical fitness and showed an increase of 1.37 times in the height of kick after exercise. Two of the group were below average in fitness and exhibited a kick 1.11 times higher than before exercise.

Subjects 6, 10, 17, 30, 31 and 43 were above average in physical fitness. From this group, subjects 6, 17 and 43 were tired from their exercise and their kicks were 1.47 times greater after exercise than before. Subjects 10, 30 and 31, who were not tired, recorded kicks 2.14 times greater than before exercise. None of those in group 1 who were average or below average in fitness were tired by the exercise which they took.

B: Subjects who showed a decrease in the extent of their knee-jerk after exercise. Fifteen subjects are included in this group, and the data obtained are shown in table 2.

The records of subject 5 are representative of this group and are shown in figures 3 and 4. The subject is 22 years old, a junior in the University, and was a candidate for a position on the varsity wrestling team. The record was taken early in the practice season.

For these fifteen subjects we find that, as a group, the height of their kicks was only 0.68 times as great as before exercise. The data further show that seven in this group were below average in physical fitness, and that their knee-jerk after exercise was 0.56 as high as before exercise. Six of this group were average in physical fitness and showed a kick 0.81 times as high as before exercise.

Subjects 3 and 35 were above average in physical fitness and registered a knee-jerk 0.71 times as high after exercise as before.

Discussion. The data for group 1 seem to indicate that the height of the knee-jerk is greatly increased by exercise so long as the sensation of

TABLE I

SUBJECT NUMBER	AVERAGE HEIGHT OF KICK		Ex/n RATIO	EXERCISE TAKEN	EXTENT OF FATIGUE	DEGREE OF FITNESS
	Before	After				
	mm.	*mm.*				
6	24.32	25.87	1.06	3 hours football	Tired	Above average
24	20.86	22.24	1.07	1 hour walk	Not tired	Average
4	17.58	19.09	1.09	½ mile run	Not tired	Below average
12	17.77	20.95	1.12	1 hour gym. work	Not tired	Average
18	18.88	21.34	1.13	1 hour janitor work	Not tired	Below average
46	17.28	20.23	1.17	¼ hour stair climbing	Not tired	Average
41	9.71	11.44	1.18	¼ hour stair climbing	Not tired	Average
10	30.48	38.29	1.25	1 hour gym. work	Not tired	Above average
29	18.75	24.78	1.32	½ hour gym. work	Not tired	Average
17	48.39	67.89	1.40	2½ hours football	Tired	Above average
34	7.27	11.24	1.55	1 hour walk	Not tired	Average
43	9.63	18.81	1.95	2 hours basketball	Tired	Above average
21	7.77	16.88	2.17	1 hour walk	Not tired	Average
31	34.72	76.83	2.21	2 hours wrestling	Not tired	Above average
30	4.27	12.75	2.95	1 hour walk	Not tired	Above average

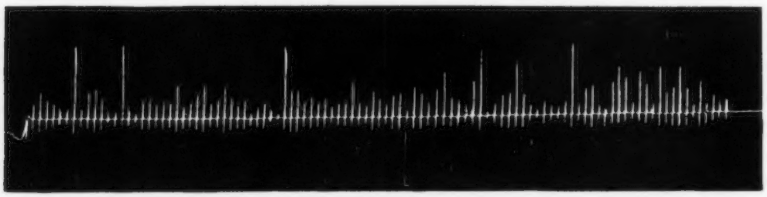

Fig. 1. Record of subject 30 taken before exercise. Average height of kick, 4.27 mm.

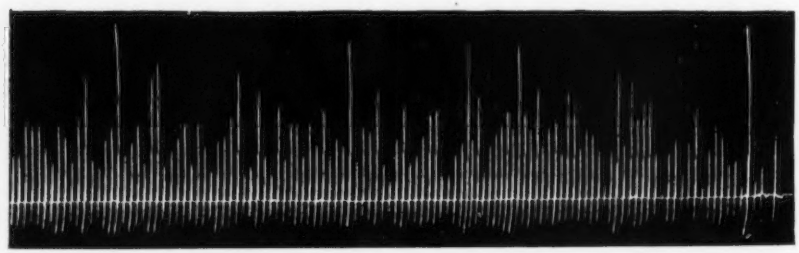

Fig. 2. Record of subject 30 taken after exercise. Average height of kick, 12.75 mm., showing an increase of 2.95 times.

TABLE 2

SUBJECT NUMBER	AVERAGE HEIGHT OF KICK		Ex/n RATIO	EXERCISE TAKEN	EXTENT OF FATIGUE	DEGREE OF FITNESS
	Before	After				
	mm.	*mm.*				
15	2.26	2.15	0.95	½ hour "dog trot"	Not tired	Average
38	28.43	26.43	0.93	¼ hour stair climbing	Legs tired	Average
39	6.00	5.49	0.92	¼ hour stair climbing	Tired	Below average
11	5.89	5.07	0.86	2 hours basketball	Tired	Average
20	43.94	37.49	0.85	½ hour walk	Legs tired	Below average
3	25.41	21.43	0.84	1 mile run	Tired	Above average
22	5.09	4.10	0.81	2 hours wrestling	Tired	Average
33	8.93	7.01	0.78	1 hour walk	Legs tired	Average
37	41.82	25.32	0.61	¼ hour stair climbing	Tired	Below average
35	37.40	21.95	0.59	2 mile run	Tired	Above average
5	9.58	5.40	0.56	1 hour wrestling	Tired	Average
25	12.07	6.57	0.54	1 hour walk	Tired	Below average
28	15.14	6.07	0.40	½ hour walk	Tired	Below average
19	17.29	6.08	0.35	1 hour walk	Tired	Below average
1	5.31	1.19	0.22	1 mile run	Tired	Below average

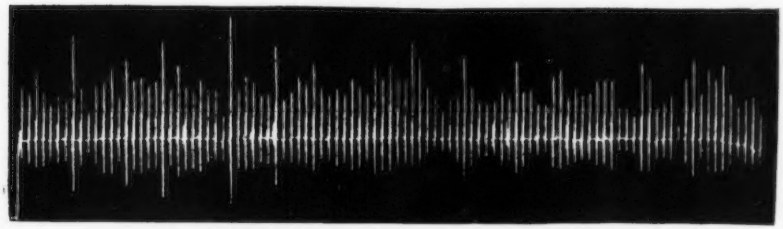

Fig. 3. Record of subject 5 taken before exercise. Average height of kick, 9.58 mm.

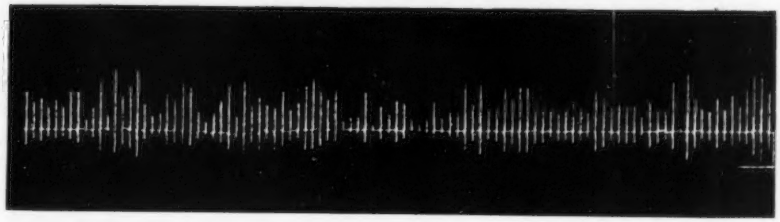

Fig. 4. Record of subject 5 taken after exercise. Average height of kick, 5.40 mm., showing a decrease of 0.56 times.

fatigue is absent. The amount of increase seems to be in direct proportion to the physical fitness of the subject, which, in turn, is a significant factor in delaying fatigue. This is shown in table 3.

The three subjects of group 1 who were reported as fatigued had been candidates for positions on varsity athletic teams for some time and were in fit condition to indulge in more strenuous and prolonged exercise. In spite of the great amount of work done by them they showed an increase in the extent of their knee-jerk.

The data for group 2 seem to indicate that the knee-jerk is decreased by exercise which brings on fatigue. The one exception to this, subject 15, showed the least decrease in the extent of his kick of any member in the group. The only explanation evident, in view of the rather consistent results shown by the others, is that he might not have been classified correctly as to the extent of his fatigue. He volunteered no statements and he did not seem tired. Those who were below average in fitness showed a greater decrease than those who were average in fitness, the ratio being 0.55 to 0.81. Subjects 3, and 35, who were above average in fitness, ac-

TABLE 3

NUMBER OF SUBJECTS	DEGREE OF FITNESS	AVERAGE Ex/n RATIO
3	Above average	2.14
7	Average	1.37
2	Below average	1.11

complished their work in a shorter length of time than others who took similar exercise.

In general those who were in fit condition for the task undertaken showed increased tonus, while those who were not in fit condition for the task undertaken showed decreased tonus. This is clearly shown when the results of those who did similar work are compared. Each of the football men was above average in fitness, and each showed an increase in the extent of his kick after exercise. The basket-ball men and wrestlers showed interesting changes. Records taken later in the practice season when the men were truly above average in fitness showed an increase after exercise in each case. Records taken earlier in the practice season, before the men had become above average in condition, showed a decrease after exercise in each case. The men who climbed stairs for fifteen minutes and those who took an hour's walk showed comparable results. Those who were in average condition were not tired and showed an increase; those who were below average were tired and showed a decrease. Subject 38, who climbed stairs, and subject 33, who took an hour's walk, were in average condition and showed a decrease after exercise. Neither of these men was generally fatigued; each reported that his legs were

tired, or felt heavy. Subject 4, who was below average in fitness, ran a half-mile at an easy gait, was not tired, and showed an increase. Subjects 3 and 1 each ran a mile. Subject 3, who was above average in fitness, made a much faster run than subject 1, was tired, and showed a decrease. Subject 1, who was below average in fitness, tried to make a fast run, was the most thoroughly tired of any of the subjects, and showed the greatest decrease in the extent of his kick. Subject 35 made fast time in his two-mile run, was tired, and showed a decrease. Subjects 29 and 12 were each in average condition. Subject 29, who took a half-hour of gymnasium work, showed a greater increase than subject 12 who took an hour of gymnasium work. Subject 10, who was above average in fitness, showed a greater increase after an hour of gymnasium work than subject 12, who was average in fitness.

These experiments confirm the findings of both Lombard and Sternberg and indicate how it is possible for both to be correct in their contradictory findings. They also seem to confirm the work of Ryan, who used an entirely different procedure.

The data seem to conform to the well-known physiological principles that "the effect of activity is in the beginning beneficial to a muscle in that its irritability steadily increases," but "after the period of the 'treppe' has passed, the contractions diminish steadily in height, until at last the muscle fails entirely to respond to the stimulus" (5). It is the intention of the writer to carry out further experiments in an attempt to determine whether or not muscular tonus as exhibited by the knee-jerk does conform to the principles of "treppe" and fatigue as applied to muscular irritability.

SUMMARY

Muscular tonus, as exhibited by the knee-jerk, is heightened by activity so long as the activity does not cause fatigue. It is decreased by activity whenever the activity is sufficient to cause fatigue.

It is a pleasure to express my appreciation to Dr. W. W. Tuttle for the suggestions which he has given me throughout this experiment.

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INFLUENCE OF GLANDS WITH INTERNAL SECRETIONS ON THE RESPIRATORY EXCHANGE

VIII. The Effect of Feeding Emulsions of the Interrenal Gland to Rabbits

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In 1921 (1) and in 1922 (2) Marine and Baumann reported experiments on 53 rabbits showing that partial but sufficient destruction of the function of the interrenal gland (suprarenal cortex) in rabbits with intact thyroids usually leads to an increased heat production lasting from a few days to several months, depending on the degree of injury produced and the rapidity of compensation. It was also pointed out that lethal destruction of the interrenal function causes a fall in heat production and that less serious injuries are usually without effect on the respiratory exchange. These observations were confirmed by Scott (3) in this laboratory, using cats.

In 1922 Marine and Baumann (4), (5) also demonstrated on 15 rabbits that removal of the thyroid gland prevents or greatly lessens the increased heat production which usually follows partial but sufficient destruction of the function of the interrenal gland.

On the basis of these findings the hypothesis was advanced that the interrenal gland normally exercises an inhibitory or regulatory influence over the activity of the thyroid gland. Our hypothetical conception of the mechanism of this regulatory action was that a substance (hormone) was discharged from the interrenal gland into the blood or lymph stream, carried to the thyroid and there exerted its regulatory effect on the secretory activity of the thyroid cells. It was also pointed out that while this thyroid-interrenal interrelationship was a striking and readily demonstrable one, there was abundant, although less objective, evidence that the interrenal gland exercises a similar regulatory function over the activity of other body tissues as well. The possibility that this substance exercises its regulatory and inhibitory activity on tissues indirectly through the visceral nervous system must be borne in mind.

Additional evidence of the influence of the interrenal gland on heat production was obtained on 10 normal babies in 1922 (6). There occurs

during the second week of extra-uterine life in babies a remarkable natural destruction of a large part of the two inner layers of the interrenal gland (the gland is very large in babies during intra-uterine life) and it occurred to us that if experimental destruction of the interrenal gland in rabbits and cats could cause an increase in heat production perhaps the same reaction occurs during the natural or normal destruction of this gland in babies. Our studies showed that it did occur. During the second week of life and concomitant with the physiological destruction of portions of the interrenal gland there occurs a sharp increase in heat production which is much greater than the combined rise of the next three weeks of life. Further evidence of the influence of the suprarenal gland on the thyroid was furnished in 1922 when Black, Hupper and Rogers (7) observed that feeding suprarenal residue (nature not stated) increased the iodin store in the thyroid of dogs. Cameron (8) in 1923 reported that suprarenal cortex when fed to white rats along with desiccated thyroid appeared to counteract the thyroid effect in the ratio of 10 to 20:1 (cortex to thyroid).

In view of these facts and the hypothesis based thereon, we undertook experiments to determine whether heat production in the normal animal (rabbit and cat) could be lowered by the administration of interrenal gland extracts. Reference has already been made to the effect of feeding fresh ox interrenal glands in cases of exophthalmic goiter (9), (10) and also of the fact that we have been able to obtain a definite reduction in the heat production of rabbits by the oral administration of glycerol extracts of fresh ox interrenal glands (11), (12). It is now our purpose to report further, though preliminary data, regarding the effect of ox interrenal emulsion[1] on heat production in rabbits. Eighteen rabbits have been studied. The principal data of these experiments are given in table 1.

The period of feeding varied from 11 to 19 days and the amounts of the emulsion administered, from 4 to 10 cc. daily. Eleven rabbits showed a fall in heat production varying from 6 to 27 per cent, while 7 showed no noteworthy change. The time interval between the beginning of feeding and the onset of the fall in metabolism, with two exceptions, ranged between 5 and 7 days. It is considered highly significant that this time interval seems to bear some relation to the time interval elapsing before the onset of the fall in metabolism in rabbits following thyroidectomy,

[1] The emulsion is made from ox suprarenal glands not over 8 hours post-mortem: that is, glands are received in the morning and the emulsion prepared during the afternoon of the same day. The preparation of this emulsion consists in removing the pericapsular fat with scissors, sagittally sectioning the glands with a razor and removing the medulla with scissors. This process removes approximately 90 per cent of the epinephrin. The cortical mass is then finely hashed and mixed with equal parts by weight of c.p. glycerol. After standing several days it is filtered through one layer of gauze to remove the coarsest particles. This may then be fed to rabbits through wide mouth pipittes without difficulty.

which is also between 5 and 7 days. This suggests that the metabolism-depressing substance acts directly, or indirectly through the visceral nervous system, on the thyroid cells either to inhibit the formation of the thyroid hormone or to prevent its excretion rather than to neutralize any secretion. In all cases the heat production increased again after stopping the emulsion. In most instances this occurred between the 4th and 8th day, but in one instance it was delayed over a month.

TABLE 1

RABBIT NUMBER	SEX	AGE FEEDING BEGAN	DATE FEEDING BEGAN	DURATION OF FEEDING	AMOUNT EMULSION FED DAILY	AVERAGE METABOLISM BEFORE FEEDING	AVERAGE METABOLISM AFTER FEEDING	PERCENTILE FALL IN METABOLISM	NUMBER OF DAYS FED BEFORE FALL BEGAN	DURATION OF FALL
				days	*cc.*	*calories per kgm. per hour*	*calories per kgm. per hour*			*days*
280	M.	486 days	10–17–22	19	4	2.66	2.43	9	7	15
281	F.	486 days	10–17–22	19	4	3.15	2.30	27	7	60
315	F.	515 days	12– 6–22	12	10	2.87	2.44	15	5	12
387	F.	8 months	12– 6–22	12	10	2.90	2.50	14	6	30
388	F.	8 months	12– 6–22	12	10	2.25	2.10	6	5	11
432	F.	7 months	12– 6–22	12	10	3.25	2.40	23	6	13
373	M.	298 days	2– 1–23	11	10	2.62	2.40	8	6	22
385	M.	277 days	1–10–23	17	5	2.85	2.30	19	8	15
392	M.	9 months	1–10–23	17	5	2.90	2.50	14	5	13
407	M.	8 months	1–10–23	17	5	2.45	2.20	10	12	8
439	M.	7 months	2– 1–23	11	10	2.60	2.20	15	7	17
363	M.	275 days	12– 6–22	12	10	2.60	2.70	No change		
366	F.	250 days	12– 6–22	12	10	3.00	2.90	No change		
405	F.	8 months	12– 6–22	12	10	2.45	2.45	No change		
406	M.	8 months	12– 6–22	12	10	2.90	2.90	No change		
383	M.	298 days	2– 1–23	10	10	2.60	2.60	No change		
444	F.	11 months	2– 1–23	11	5	2.45	2.45	No change		
445	F.	7 months	2– 1–23	11	5	2.50	2.50	No change		

Two types of control experiments were used; 1, rabbits without, and 2, rabbits with glycerol feeding. In none of these experiments was a comparable fall in heat production noted.

All animals used were adults—over 7 months of age. This is important since the rate of metabolism steadily falls in rabbits (as in other mammals) from birth to puberty (about the 5th month). It then falls more gradually until between the 6th and 7th months, when often another slight but abrupt drop in the metabolic rate may occur.

We have as yet no explanation for the seven experiments in which inter-

renal gland emulsion failed to cause a fall in heat production. It is possible that for some reason these were more resistant or that none of the substances escaped destruction in the alimentary tract or that some counter mechanism was in operation to offset any action of the interrenal emulsion or that the emulsion used was inactive. It may be pointed out that epinephrin has pharmacological activities which could permit it to function as an antagonist of the metabolism-depressing substance in the cortex.

The feeding of glycerol emulsions by mouth is a very crude method of attempting to influence living cells with substances which are normally discharged directly into the blood or lymph stream for this purpose because such substances are exposed to all the hazards of destruction by the digestive processes. Thyroid gland is at present the only opo-therapeutic agent that is highly effective when so administered, although Uhlenhuth (13) has obtained glimpses of the growth-promoting principle of the anterior pituitary by feeding it to salamanders. His results, however, are not comparable with the results obtained by Evans and his collaborators (14) from injecting aqueous extracts of the fresh gland intraperitoneally into rats.

What we have observed in rabbits and in cases of exophthalmic goiter, therefore, is only a glimpse of what we believe will be obtained when this metabolism-depressing substance is sufficiently concentrated and isolated to permit of its parenteral introduction.

Dried preparations, both commercial and those prepared by ourselves, in our hands have been without definite effects as have also been a large number of extracts prepared during our attempts to concentrate this substance for subcutaneous or intraperitoneal injection. Many of these extracts have contained more or less epinephrin and had a tendency to raise metabolism when injected subcutaneously.

SUMMARY

We have observed a decrease in heat production following the oral administration of glycerol emulsions of fresh ox interrenal glands in 11 of 18 rabbits. This fall in heat production usually begins in from 5 to 7 days after feeding has begun and persists in the majority of cases from 4 to 8 days after the feeding is stopped. It is believed these results represent only a glimpse of what may be obtained when the metabolism-depressing substance is sufficiently isolated and concentrated to be introduced into animals parenterally.

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CONCERNING THE MODE OF TRANSFERENCE OF CALCIUM FROM THE SHELL OF THE HEN'S EGG TO THE EMBRYO DURING INCUBATION

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One of the authors (Buckner) of this paper, while working at the Pasteur Institute in Paris, France, subsequent to a discussion concerning the calcium metabolism of the laying hen with Prof. E. Fourneau, conceived the idea of determining, if possible, the mode of transference of calcium from the shell of a hen's egg through the shell membranes to the embryo during incubation. Delezenne and Fourneau (1) have published detailed results concerning the quantitative transference of calcium from the shell to the embryo during incubation and have shown that the calcium increase in the embryo is progressively greater as the stage of incubation advances and particularly is this true from the 17th to the 21st day. They have also shown that the content of calcium in the liquid portion of a freshly laid egg is 0.0354 gram, calculated as CaO, which increases approximately 500 per cent during incubation.

Atwood and Weakly (2) have shown that fertile eggs give off approximately 10 grams of carbon dioxid during incubation, in daily increasing amounts, the increase being especially marked during the last few days.

Lawson and Edmond (3) conclude that the embryo is the chief source of carbon dioxid in incubation and that the carbon dioxid given off increases during the incubation period, with the exception of slight falls on the 1st and 16th day, and that after the 3rd day the increase of carbon dioxid is proportional to the increase in weight of the developing embryo.

With the view of throwing more light on the subject, a freshly laid White Leghorn hen's egg weighing 54 grams was selected and a small hole broken in the large end of the shell at the air chamber, care being taken not to break the inner shell membrane. The shell was carefully removed in small pieces until the hole was $1\frac{1}{2}$ cm. in diameter. The opening was then washed with a stream of distilled water to remove any small, loose pieces of shell. Then a hole was cut in the inner membrane so that the membrane extended beyond the shell, and the yolk and white were drained into a dish. The interior of the shell was washed carefully with distilled water and 30 cc. of distilled water placed inside the shell. A stream of carbon dioxid was

passed slowly through the water in the egg shell for 4 hours, at 24.5°C. at the rate of 1 cc. per second. The clear water was then removed and tested. The hydrogen ion[1] concentration of this solution was pH 6.8 as contrasted with pH 8.3 for the distilled water before the CO_2 had been passed through it, duplicate tests giving the same result. On boiling, it gave a white precipitate, a portion of which, tested with dilute acetic acid, was found to be easily soluble, with effervescence. The rest of the precipitate was filtered out and ashed. The ash effervesced with acetic acid and a white precipitate was formed when ammonium oxalate was added to the solution, showing the presence of calcium. These tests show the presence of calcium bicarbonate in the carbonated water in the egg shell.

The experiment was repeated with two other eggs and the water, when removed, was evaporated to dryness separately, the residues ashed and calcium determined according to McCrudden (4). The results showed that the water contained 0.020 per cent and 0.022 per cent of calcium oxid, respectively, or 0.0060 gram and 0.0066 gram in the whole quantity of water from each egg shell, equivalent to 0.0107 gram and 0.0118 gram of calcium carbonate.

In another egg shell, egg white was used instead of distilled water, and carbon dioxid was passed through it for 4 hours, at 24.5°C., but owing to loss of much of the albumen by foaming, no quantitative determination of the calcium content was attempted. When the part remaining was evaporated to dryness and ashed, the ash effervesced with dilute acetic acid and showed an appreciable amount of calcium when precipitated as the oxalate. The hydrogen ion concentration of this solution was pH 6.8 as compared with pH 8.4 for egg white that had remained in the air for a similar period.

Four other egg shells were prepared in like manner and 20 cc. of the mixed whites put into each. Two of these were put into a Novy jar which was then filled with CO_2 at atmospheric pressure; the other two were placed in a Novy jar containing air. The two jars were allowed to stand at 24.5°C. for 12 hours, when they were refilled with the appropriate gases and allowed to stand 12 hours longer. The albumen in each shell was removed and analyzed separately. The analyses of the egg white which had stood in the atmosphere of carbon dioxid gave 0.0057 gram and 0.0055 gram of calcium oxid in the whole quantity, respectively, or 0.028 and 0.027 per cent. This is equivalent to 0.0102 gram and 0.0098 gram of calcium carbonate, respectively. The controls, or the ones in the atmosphere of air, gave 0.0019 gram and 0.0015 gram of calcium oxid, or 0.0095 and 0.0075 per cent, equivalent to 0.0034 gram and 0.0027 gram of calcium carbonate. When the average, 0.0030 gram, for the quantity of $CaCO_3$

[1] The hydrogen ion concentrations were made colorimetrically by Dr. D. J. Healy, of this Station.

in the 20 cc. of egg white in the atmosphere of air is subtracted from the 0.0100 gram of calcium carbonate, the average amount found in a similar quantity in an atmosphere of CO_2, 0.0070 gram of calcium carbonate remains, the amount dissolved from the shell by CO_2. The hydrogen ion concentrations were pH 6.0 and pH 8.4, respectively, for the egg white which had been in atmospheres of CO_2 and air.

These experiments give us proof that water containing carbon dioxid passes through both the egg shell membranes to reach the shell and that calcium is dissolved by it from the shell, as calcium bicarbonate which, by diffusion, passes back through the membranes into the water or egg white contained in the shell.

We infer that during the first 9 days of incubation, before the allantois has touched the shell membrane, a water solution containing carbon dioxid given off by the embryo diffuses through the white and the membranes to the shell, there forming calcium bicarbonate which diffuses back to the embryo, where it is metabolized. After the ninth day, when the allantois touches the shell membranes, it is reasonable to believe that the shell then gives up calcium bicarbonate to the blood stream as it discharges carbon dioxid.

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THE INFLUENCE OF INSULIN ON THE INORGANIC AND ORGANIC PHOSPHATES OF THE LIVER

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The changes in the phosphates of the muscle during insulin action have been studied by Harrop and Benedict (1), Audova and Wagner (2) and by Kay and Robison (3). These authors found an increase in "lactacidogen" phosphorus of the muscles of insulinized rabbits, while the inorganic phosphates were not changed. However, Sokhey and Allan (4) stated that they were unable to verify the above results as far as the lactacidogen was concerned. It should also be noted that Embden, Schmitz and Meincke (5) were unable to detect an appreciable increase in the lactacidogen content of the muscles of rabbits and dogs which were fed for several days with large amounts of sugar and phosphates.

It seemed of interest to study what changes would occur in the phosphates of the liver, especially in view of the decrease of the free liver sugar during insulin action, a phenomenon which has been described on former occasions (6), (7). Could the strong diminution in the free sugar of the liver be explained by the formation of a hexose-phosphoric acid complex? There is no doubt that changes in the phosphates are in some way connected with carbohydrate metabolism, as can be seen from the work of the first four authors cited above and from that of Wigglesworth, Woodrow, Smith and Winter (8), Briggs, Koechig, Doisy and Weber (9), Perlzweig, Latham and Keefer (10), Blatherwick, Bell and Hill (11), Allan, Dickson and Markowitz (12) and others. However, the writers are inclined to believe that the evidence so far produced is too scanty to assume definitely that a hexose-phosphoric acid complex is formed during insulin action. If it were formed, it would certainly not account for all the sugar that disappears during insulin action.

Embden and Laquer (13) identified "lactacidogen" as a hexose-diphosphoric acid complex, which they were able to isolate from striated muscle, but not from smooth muscle. The isolation from other tissues than muscle was apparently not attempted, so that it is undecided whether or not a hexose-diphosphoric acid is present in the liver. The method for the quantitative determination of lactacidogen phosphorus, as worked out by Embden, Schmitz and Meincke (5), is based on the fact that this substance,

probably under the influence of a ferment, splits off phosphoric acid very rapidly (with liberation of an equimolecular amount of lactic acid). When macerated muscle tissue is incubated at body temperature, preferably in a bicarbonate alkaline medium, the liberation of phosphoric acid is completed in one to two hours. By substracting the inorganic phosphates originally present, which are determined in a separate portion of the muscle under special precaution against post-mortem disintegration of the lactacidogen, from the total amount of inorganic phosphates formed during incubation, the lactacidogen phosphorus is obtained. The liver, according to our experiments, also contains an organic phosphorus compound, which liberates its phosphoric acid very rapidly during incubation at body temperature. The liberation is practically completed in two hours and reaches values which do not differ materially from those obtained from muscle under similar experimental conditions.

EXPERIMENTAL. Full-grown male and female mice from the same strain were used. One control mouse was always killed as simultaneously as possible with one injected mouse, the insulin (0.08 to 0.16 unit) being given intraperitoneally. When inorganic phosphates were determined, the mice were stunned by a blow on the head, the liver removed as quickly as possible and immediately frozen in a stream of compressed CO_2. Fourty to 60 seconds elapsed from the moment of killing until the liver was frozen. The liver was then weighed in the frozen state, ground up with ice-cold 2 per cent HCl and transferred to a 10 cc. volumetric flask, 5 cc. HCl being used in all. The flask was made up to the mark with 5 per cent $HgCl_2$. On the following day the protein precipitate was filtered off, the filtrate freed from mercury and H_2S and the phosphorus determined by the Briggs (14) modification of the Bell and Doisy method. Care was taken to adjust the standard to the same acidity as the unknown. In the case of the organic phosphates, the liver was ground up with 3 cc. of a 1 per cent $NaHCO_3$ solution and incubated at 37°C. After neutralization, addition of HCl to make 2 per cent and precipitation of the proteins with $HgCl_2$, the samples were treated as under inorganic phosphates. The free sugar was determined by the Hagedorn and Jensen method (15) in the same filtrate that was used for the phosphorus determination.

DISCUSSION. Table 1 shows that insulin produces a slight, but distinct rise in the inorganic phosphates of the liver, while the free sugar of the liver, as has been described previously, is lowered by insulin. Table 2 shows that the amount of organic phosphates liberated during incubation is slightly greater in the liver of the insulinized mice than of the controls, the difference being most marked after an incubation time of one half and one hour. This might be attributed to a greater lability of the organic phosphates, which would also explain the slight increase in the

inorganic phosphates. It seems possible that the profound changes in the liver produced by insulin, especially the rapid decrease in the glycogen might increase the tendency of the very labile organic phosphorus to disintegrate into its components. However, after an incubation time of two and three hours the difference in the amount of phosphorus liberated in

TABLE 1

The influence of insulin on the inorganic phosphates of the mouse liver

The values are given in grams phosphorus per 100 grams of fresh tissue

INORGANIC P	FREE SUGAR	REMARKS
per cent	*per cent*	
0.033	0.136	Average of 14 experiments on insulinized mice*
0.028	0.298	Average of 14 experiments on control mice*

* Seven mice each starved for 1 hour, 3 mice each starved for 3 hours and 4 mice each starved for 18 hours prior to the experiments. Average starvation time, 6 hours.

TABLE 2

The influence of insulin on the organic phosphates of the mouse liver

The animals were starved from 1 to 3 hours previous to the experiments. The average values for the inorganic phosphates in table 1 (0.033 for insulinized mice and 0.028 for controls) have been substracted in each case from the total phosphates obtained during incubation. In this way the values recorded in this table are made to correspond to the lactacidogen phosphorus fraction of Embden. All values are given in grams phosphorus per 100 grams fresh tissue.

LENGTH OF INCUBATION AT 37°C.	INSULINIZED MICE		CONTROL MICE		REMARKS
	Organic P	Free sugar*	Organic P	Free sugar*	
hours	*per cent*	*per cent*	*per cent*	*per cent*	
½	0.070		0.061		Average of 7 mice each
1	0.118	0.690	0.105	1.68	Average of 6 mice each
2	0.144	0.593	0.141	1.24	Average of 5 mice each
3	0.145	0.583	0.143	2.14	Average of 5 mice each

* The free sugar values in this case correspond to the actual free sugar content of the liver plus the amount of sugar set free by the hydrolysis of the glycogen during the incubation. Since the liver glycogen of mice is hydrolyzed very rapidly at 37°C., the values are roughly an indication of the glycogen content of the liver at the time of killing. It will be noted that insulin reduces the liver glycogen of mice, which is in harmony with previous observations (16).

the liver of insulinized and of control mice was practically negligible. An increase of 3 mgm. (2 hours' incubation) would correspond to about 5 to 6 mgm. glucose if a hexose-diphosphoric complex were formed. Table 1 showed that the reduction of the free liver sugar amounted to 162 mgm., hence it can be concluded that the free sugar of the liver does not diminish on account of entering into a combination with phosphoric acid.

CONCLUSIONS

The decrease in the free sugar of the liver, observed with great regularity during insulin action, is not due to the formation of a hexose-phosphoric acid complex in this organ.

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AMINO ACIDS IN NUTRITION[1]

IX. The Rôle of Alanine and Indol in the Synthesis of Tryptophane by the Animal Organism[2]

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Since McCollum, Simmonds, and Parsons had demonstrated that proteins of the cereal grains fail to supplement each other (1), and since cystine had been shown to be a deficient amino acid in a large number of leguminous seeds (2), the possibility that cystine is a common deficient amino acid in cereal grains was anticipated. The original purpose of this investigation was to find out whether cystine is a determining growth-limiting factor in the proteins of corn. This, however, was not found to be the case. On the other hand, as previously reported by Hogan (3), tryptophane showed itself up to be the primary growth-limiting factor in the proteins in question. Because of the remarkable responses in growth obtained with tryptophane (which is indol-alanine), it was thought that it might be of some interest in physiology to study the possible synthesis of tryptophane by the animal organism from its two derivatives, namely, indol and alanine.

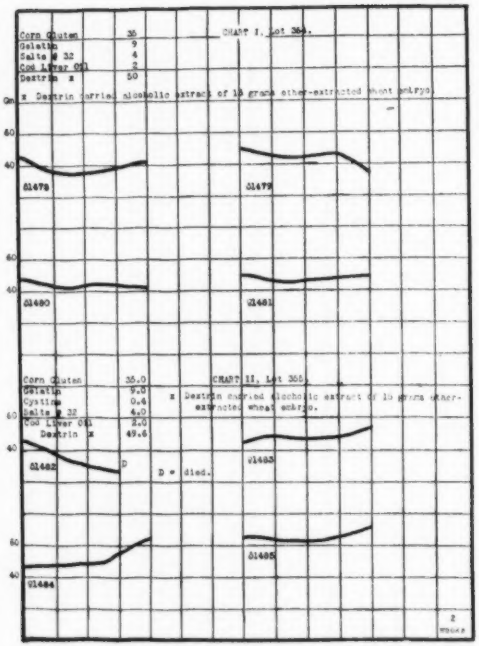
Ward (4) studied the metabolism of indol-propionic acid. This substance was dissolved in sodium bicarbonate solution and injected subcutaneously into a rabbit. The constitution of the substances found in the urine was studied by the quartz untra-violet spectraphotometer, and the conclusions reached by the author were reported as follows (4):

"Indol-propionic acid in its passage through the animal body appears to undergo oxidation in the a-carbon atom of the pyrrole ring with formation of an a-hydroxy-indol compound, which may be either in the enol or keto form." Whether that same substance might serve as a nucleus for building of tryptophane when taken in orally, in a ration satisfactory with respect to all dietary factors with the exception of the amino acid under consideration, has not hitherto been tried.

A. E. Taylor (5) obtained synthesis of a protamine by the action of

[1] Research paper no. 12, Journal Series, University of Arkansas.

[2] An abstract of this paper was presented before the American Society of Biological Chemists, at St. Louis, December 27, 1923.



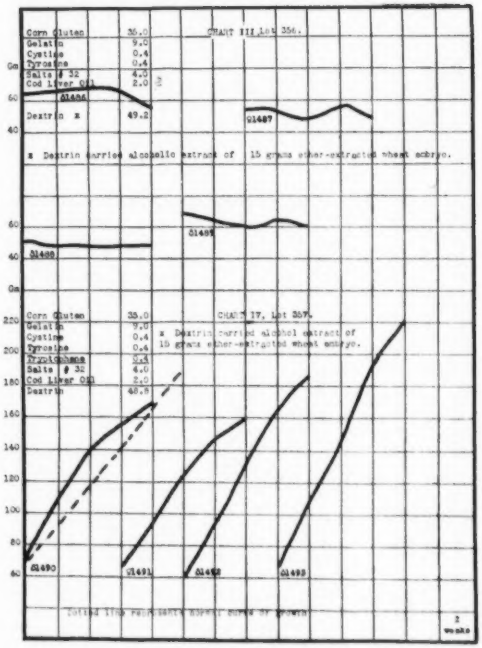


Chart I, lot 354. This chart shows that gluten feed, as a source of total corn proteins, fed at a 9 per cent level, even in the presence of gelatin, fails to support growth.

Chart II, lot 355. The addition of cystine to the ration consumed by lot 354 did not result in any response in growth. It is, therefore, concluded that cystine is not a determining growth-limiting factor in the proteins of corn.

Chart III, lot 356. This experiment had the same composition as lot 355, with the exception of its further fortification with 0.4 per cent tyrosine. Only maintenance was secured on this ration, showing tyrosine also not to be a deficient amino acid in the proteins under investigation.

Chart IV, lot 357. The addition of 0.4 per cent of tryptophane to a 9 per cent corn protein diet, in the presence of cystine and tyrosine, produced normal growth. Since Hogan had already demonstrated tryptophane to be a deficient amino acid in corn proteins, the response of that amino acid in this experiment is to be credited to the proteins of corn rather than to gelatin.

trypsin on the products of a tryptic digestion of the same substance, and T. B. Robertson (6) obtained "paranuclein" by the action of pepsin on the products of a prolonged peptic digestion of casein. It was, therefore, thought not at all impossible that the animal organism might have the

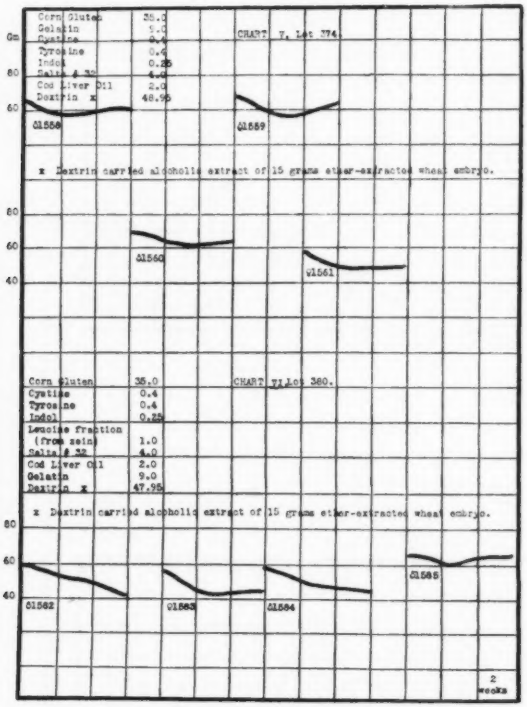


Chart V, lot 374. Although indol is an odoriferous substance, and is generally considered as a toxic end product of protein metabolism, resulting from bacterial putrefaction in the large intestine, that substance in the concentration employed did not manifest any external noticeable injurious effect on the rats. The ration was eaten liberally, but no response in growth was obtained.

Chart VI, lot 380. As a source of alanine, the leucine fraction, high in alanine, from an acid hydrolysis of zein, was employed. The addition of indol and alanine in this ration did not replace tryptophane.

synthetic power of utilizing indol, an end product of protein metabolism, when administered orally simultaneously with alanine, or a-amino-propionic acid, toward the construction of the indispensable amino acid, tryptophane (7).

EXPERIMENTAL. As a source of corn proteins, corn gluten feed, having a total protein content of 25.7 per cent, was used. It was calculated that, in order to introduce 9 per cent protein in the ration, it would be necessary to feed the gluten product (purchased from the Keever Starch Co., Columbus, Ohio) at a 35 per cent level. The results of the experiments are indicated in the accompanying charts.

SUMMARY

1. Tryptophane is the primary growth-limiting factor in the proteins of corn.

2. The animal organism[3] does not possess the capacity of synthesizing tryptophane from its two derivatives, namely, alanine and indol, when ingested orally.

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[3] In this investigation, as well as in previous work of a similar character, the albino rat was the experimental animal employed.

THE MAXIMUM OF HUMAN POWER AND ITS FUEL

From Observations on the Yale University Crew, Winner of the Olympic Championship, Paris, 1924

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The maximum physical power, of which a man in the prime of his strength and at the height of athletic training is capable, is a matter with many important bearings. It is probable that the rowing of a crew in a racing shell with sliding seats is a form of exercise in which a greater total energy expenditure is attainable, for periods of five to twenty minutes, than under any other conditions. No other exertion comes so near to bringing the entire muscle mass of the body into maximal extension and contraction. Any form of work or exercise involving a smaller part of the musculature limits total power through the protective sensations of fatigue in the part exerted. What is termed "wind"—the breathing, sensations from breathing, and the associated rapidity of the heart beat—also sets a limit which, if estimated in total power, is much lower for small than for large muscle masses; much greater respiratory distress may result from overworking a few muscles, than from the same energy expenditure when more widely distributed and less intense.

No form of exercise involving only the arms and shoulders—or even the arms, shoulders and back, as in a boat with a fixed seat (1)—can reach a very high total power. An exertion using merely the legs, as in bicycling (2), can attain a greater total than one for the arms, for the muscles of the legs are larger than the arms; but merely to hold the body and arms rigid, as on a bicycle, involves relatively little energy. In running it is the legs that are chiefly exerted; the arms are swung, but the call on the muscles of the upper limbs is comparatively small; the trunk is bent at the waist, but as a whole, rigidity is the chief contribution of its muscles to running. Even in the exertion of stair or hill climbing, which is known to allow a large power to be brought into play, the mass of active muscle is much smaller than in the stroke used in a racing shell.

This stroke begins in extreme flexion of trunk and legs, with a powerful drive of the extensor muscles, the strongest chain of muscles in the body; it then passes rapidly to extreme extension, pulling throughout against

the high resistance of the oar, and ends with a powerful flexion of the arms. From this position the recovery involves a rapid bending of the wrists, lowering the hands and shooting them forward, and a bending of ankles, knees, hips, waist and shoulders, with contraction of practically all the flexor muscles to these joints. Thus the greatest possible work is obtained from the extensors of the trunk and legs and from the flexors of the arms during the pull, while during the recovery the flexors of trunk and legs and the extensors of the arms, although less heavily loaded, are yet made to pass through a maximal contraction and extension. This is repeated thirty or more times a minute.

Unusual record of the crew studied. If this form of athletics allows a development of total power beyond any other, then we may fairly claim that the data to be here presented afford at least an approximate indication of the maximum that the human engine can attain in any known form of exertion. The Yale University crew which we had the opportunity to study during the winter and spring of 1924 had an unparalleled athletic record. On May 3, it won decisively over the crews of the universities of Pennsylvania and Columbia in a race of one and a half miles on the Housatonic River; time, 8 minutes and 18 seconds. On May 17, it won as decisively over Princeton and Cornell on Lake Carnegie; distance one and three quarter miles, time 9:45⅘. The Yale crew usually trains primarily for the annual four mile race with Harvard. Late in the season, however, it was entered for the trials for the selection of the American representative in the Olympics. These races were rowed on the Schuylkill on June 13 and 14. In the first heat the Yale crew won against the Navy varsity, Navy junior varsity and the crew of the Undine Barge Club. In this race the record for the course was lowered from 6:12½ to 6:9⅘. Next day in the final, in a very hard contest, the Yale eight won over the Navy officers crew and Pennsylvania, lowering the time for the course of one and a quarter miles to 5:51⅘. This is a speed of 12.42 miles an hour, or 1092 feet per minute, a mile in 4 minutes and 50 seconds. Six days later, this crew won against Harvard, covering the four mile course at New London easily in 21:58. It sailed for France the next day, but maintained training during the voyage. At Paris it went successfully through the preliminary heats of the races on the Seine, and in the final race on July 17 won the Olympic championship with a distinct demonstration of superiority over the competing crews from all parts of the world. It led its speediest rivals by five boat lengths at the end of the 2000 meter course; time 5 minutes and 51 seconds, the world's record.

It is doubtless true that skill, and not mere strength, wins races. But it is also highly probable that men with such a record stand virtually at the acme of dynamic efficiency.

Measurement of power required to drive boat. In such a prime mover

(to use the engineering term) as an oarsman, it is easier to determine the total energy liberated within the body, than it is to estimate the percentage of this power expended in external work. The former is accurately measured by a determination of the volume of oxygen consumed from the air; but the external work is less simple. Thus the oarsman does work in moving the slide, on which he sits, back and forth along the boat during each complete stroke, and in swinging his head and shoulders. But this work does not propel the boat; indeed the primary aim during the recovery is to slide aft so smoothly that the velocity of the shell through the water is maintained; for the eight oarsmen together weigh five times as much as the boat and coxswain, so that the dead weight recoils relatively a five-fold distance and in opposite direction to every movement forward or aft on the part of the live weight.

The effective work in driving the boat is only that of the oar blades in the water, and a part even of this is lost; for the water around each oar is moved backward as the boat is driven forward. Most of the energy at this point is, however, effective; and fortunately this effective work is easily measured.

For this measurement, a racing shell containing a crew averaging 172 pounds, which is a little less than the men in the varsity boat, was towed at the end of a long line by means of a fast motor launch. To a bridle in the launch was fastened one end of a spring balance—such a balance as is commonly used to weigh ice; to the other end of the balance the tow line was made fast. Thus when the motor boat was towing the shell and crew, the heft, or so-called "draw bar pull," was weighed, and the faster the boats went the greater, of course, was the pull. Uniform speed for a measured distance was essential in each test, and after a little adjustment it was attained for quarter-mile stretches on the smooth, and at the time nearly currentless, water of the Housatonic River course; and determinations of the draw bar pull were made at a sufficient range of speeds to permit plotting a curve (fig. 1).

Racing speeds over still water lie between a mile in 5.5 minutes, as in a four-mile race, and a mile in 4.8 minutes for a mile and a quarter. The pull, as shown by the curve (see figure), rises from 98 pounds to 110 pounds for this range of speeds. Taking the pull at 110 pounds, and the speed as 1092 feet per minute, we have (1092×110) 120,120 foot pounds per minute as the absolute net efficient work done by the eight oarsmen, or 15,015 foot pounds per man per minute. This is an amount of work about equal theoretically to that of climbing an eight-story building each minute of a race.

We have devoted some effort to estimating the additional work expended, and lost, in the movement of water around the oar blades, the movement corresponding to the "slip" of a screw propeller. The blade

describes an arc of more than 90 degrees, and thus at first pushes water rather sharply outward from the boat and then toward it again, but outward much more than inward; both movements waste power. In relation to the boat the blade of each oar moves backward during each stroke about 12 feet. In relation to any chance floating speck or other object on the water the blades move about one and a half feet. This slip of the oar blade through the water, the slowing of the boat at the end of recovery

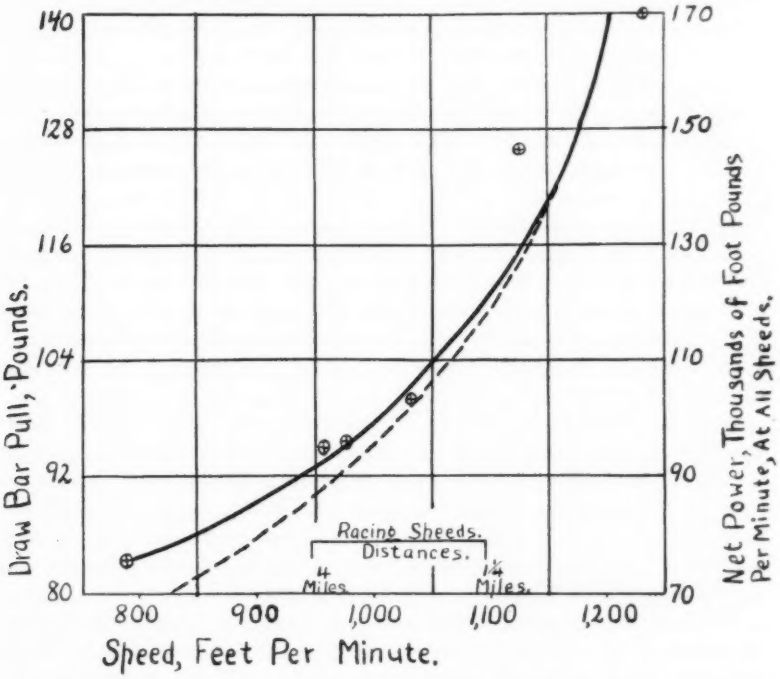

Fig. 1. Showing relations of draw bar pull to speed (solid line), and of net power to speed (dotted line) when a racing shell and crew are towed.

and the jump at the catch, the friction at oarlocks and slide, the energy expended in the recovery between strokes, and the flexing of the body and legs in preparation for another stroke,—all these factors together can scarcely amount to less than 25 per cent of additional work. Perhaps this figure should be yet larger; but even so, the estimated total per man rises thus to 18,769 foot pounds, or nearly 0.57 of a horse power, at a speed for the boat approximating that of the world's record for a mile and a quarter race. The heat equivalent for this amount of work is 6.0 calories per minute.

We shall have occasion to use this figure, 6.0, later in this paper as a check on results by quite different methods. In fact the measurements of the draw bar pull at racing speeds, as a basis for estimating the work of rowing, were made after all the other lines of investigation here reported were already complete.

Work measured on rowing machine. The ordinary rowing machine, used when for any reason it is not possible to go out in a boat, consists of a dash pot filled with glycerine. The pull on the handle, or "oar" of this apparatus forces the glycerine through small holes. We did not succeed in modifying such an apparatus to serve as a work meter.

Instead of this, another form of apparatus was developed in which the "oar" worked a pump and forced water against a resistance. The area of the piston was 5 square inches; the pressure against it during a stroke was 225 pounds per square inch, but nothing during the recovery; and the stroke of the piston was 4 inches. The work per stroke was, therefore, 375 foot pounds, or if we add 25 per cent for friction, 469 foot pounds, or in its heat equivalent 0.15 calory. The man under examination sat upon a sliding seat and used the same length of stroke with the oar, and the same movements of body, legs and arms that he would in a boat. If he rowed thirty strokes a minute, the work done on the machine was estimated at 4.5 calories; at 35 strokes it was 5.25; and at 40 strokes per minute 6.0 calories per minute.

Graphic records were made of the movements of the slide and oar. Such tracings might be useful to a man learning to row a particular type of stroke; for he could repeatedly compare his own performance with a type record of the stroke. Deviations in the catch, unevenness of pull, and movement of the slide during the recovery stand out in such records; they might save many verbal directions from the coach, and prevent the formation of bad habits, which are difficult to overcome later.

Total energy expenditure measured by respiratory exchange. Every liter of oxygen absorbed from the air by the lungs, and consumed in the body, produces 4.8 calories (with a maximum variation of 7 per cent on different diets), which in work is the equivalent of (425 $\times$ 4.8) 2040 kilogram meters, or 14,736 foot pounds. This is the gross amount of energy per liter of oxygen, out of which, it is believed, the dynamics of muscle permit only about one-third to be utilized in tension on the bone levers, while the remainder is dissipated as heat. A determination of the amount of carbon dioxide simultaneously eliminated throws light on certain internal processes of great significance.

For determining the respiratory exchange—the oxygen consumption, CO_2 production, and total volume of air breathed—respiratory apparatus was installed, along with the rowing machine above described, in a room of the University Health Department building. A spirometer with a

capacity of nearly 400 liters, and two large Douglas bags were attached by means of iron pipe of one inch bore with T. joints and shut-off valves so that the air expired could be collected for definite periods, measured, sampled and analyzed. (For the air analyses a Henderson-Orsat apparatus was used; the type now common in student laboratory courses is quite accurate enough for such work and much less trouble than a Haldane or Henderson-Haldane, or any other mercurial instrument.)

The subject wore a clip to close the nose, and breathed through a mouth piece with an inspiratory valve admitting room air, and an expiratory valve connected by a light, wide bore, corrugated rubber tube, a meter in length, to the iron pipe leading to the bags and spirometer. Although these valves were of the largest size generally used for such investigations, the air ways were somewhat too small, and during the heaviest exercise the breathing, especially during expiration, was somewhat impeded.

The man to be tested removed his outer clothing, and after adjusting the foot board sat quietly on the sliding seat of the rowing machine for ten minutes. After this partial rest his expired air was collected for five minutes. He then rowed at a uniform rate for five minutes, or in a few cases for a shorter period. The whole expired air for this period was collected in the spirometer, and the volume for each minute was noted. After this work period he again sat still for 15 minutes, while the expired air was collected in a Douglas bag for a two-minute period; then in another bag for a three-minute period, and then again for ten minutes in the spirometer, which by this time had been emptied of its previous collection. Five complete analyses were thus required for each experiment.

As observations were begun in January, long before the members of the final crew were selected, data were obtained on a number of men merely in the rowing squad, on some who later rowed in the minor crews, on two members of the 150 pound crew, and on five of those ultimately selected for the varsity eight. In only a few of the tests was there an effort to approximate the maximum power. Usually an exertion fairly equivalent to that during a practice row was all that the man was asked to make. The basal metabolism was not determined; but for men of their build, height and weight (180 to 185 pounds and from 5 foot 11 inches to 6 foot 4 inches in height, average 6 foot $1\frac{1}{2}$ inches) we can deduce from the DuBois rule that the oxygen consumption under basal conditions would be about 0.29 to 0.31 liter, and the energy expenditure therefore 1.4 to 1.5 calories per minute. We recognized that the most willing and cooperative oarsman could not, in cold blood and between two college lectures, drop in at our room, and in five minutes develop as great a power as when rowing on a crew and striving to win the Olympic championship, or to beat Harvard. Every element can be supplied artificially in experiments like these, except one—intense combative emotion.

It is just here that the determination of the draw bar pull, when the boat was towed at racing speed, has its importance; for it may fairly be assumed that the relation of the external power, thus determined as necessary for this speed, to the (hitherto unknown) oxygen consumption and energy development during a race, is nearly the same as that of the external work to the volume of oxygen absorbed and the total internal energy developed on a rowing machine in the laboratory.

Volume of air breathed and oxygen consumed. The significant data from the respiratory experiments are given in tables 1, 2 and 3. As seen in table 1, the volume of air breathed by the men of the varsity crew during the preliminary rest was 7.5 to 13 liters per minute. During the first minute of rowing they breathed 26 to 53 liters of air, and during the later minutes of this period the volume increased to a maximum of 82 liters. When they stopped rowing the volume of breathing fell rapidly; even in the first two minutes of rest it dropped to about half of that during work, and thereafter it decreased progressively, so that at the end of fifteen minutes' rest it was practically back to the volume noted before work. This capacity to "recover the breath" quickly after a great exertion, and the interior readjustments which it indicates, are definite qualifications of athletic power. (The air volumes in the tables have all been reduced to 0°C.; the barometer at New Haven is that of sea level.)

In general the amounts of carbon dioxide exhaled and of oxygen absorbed were maintained in nearly the same proportion to each other and to the volume of air breathed during work and recovery as during rest. Consequently the percentages of CO_2 in the expired air and of the oxygen absorbed show strikingly little variation, during the period of heavy work and of subsequent rest, from the figure noted during the preliminary rest. This also is a definite athletic qualification; for a man who is out of condition or over-exerted tends to blow off an excessive amount of carbon dioxide, and the percentage of this gas in the exhaled air may then fall very low during the recovery period. This occurred in none of these men. In even the severest exertion made in these tests (see table I, May 29, B.M.S.) the man was neither exhausted nor excessively winded; he could have continued the work for several minutes longer, and doubtless for a total period of 6 or 7 minutes, as in a race. (The figures given for oxygen consumed are true per cents after correction for the decrease in the volume of the air breathed.)

The points just mentioned are shown even more strikingly by the figures for the respiratory quotient, figures obtained by dividing the percentage of CO_2 by the percentage of oxygen. The respiratory quotient is significant in two respects: *a*, as to the nature of the food material being consumed at the time for fuel, and *b*, as to the accumulation in the body of incomplete combustion products and other substances tending to over-

stimulate respiration. In untrained men the latter effect sometimes causes such a marked excess of elimination of carbon dioxide over the oxygen consumed, that the respiratory quotient rises during and immediately after exertion to a figure far above the resting value, and falls later correspondingly below it. In these oarsmen, on the other hand, with a respiratory quotient approximating 0.8 during rest, the respiratory quotient in no case appreciably exceeded 0.9 during rowing nor 1.1 immediately thereafter. In other words, as machines their bodies quicken all their processes much more nearly in uniform relation than in non-athletes.

In this respect our observations differ from those of Hill (3). We are inclined, therefore, to suspect that the large displacement of CO_2 from the body, which he has observed during work, and its replacement afterward, are not primarily indicative of the process of muscular contraction, but rather of overbreathing induced by incomplete oxygenation of the arterial blood. If the latter suggestion is correct we should expect to find that inhalation of oxygen during muscular work would relieve the strain on respiration but not greatly increase the amount of immediately expendable energy. The effects of oxygen inhalation during work, as observed by Hill and particularly by Briggs (8), seem to accord with this conception.

Fuel of work. The respiratory quotients for the whole period of rowing and rest afterward show the relation of the total amount of carbon dioxide produced to oxygen consumed, and indicate the character of the fuel material burned in the muscles of the oarsmen to liberate energy. As is well known, a respiratory quotient of 0.7 indicates that the material used as fuel is fat, either from the food or, during starvation, from the tissues of the man himself. On the other hand, a respiratory quotient of 1.0 is indicative of the consumption of sugar and no fat. Normal persons on an ordinary mixed diet generally show a respiratory quotient of about 0.8; and during rest these oarsmen approximate this value. There has recently been considerable interest as to the nature of the fuel material consumed by muscle during activity arising from the work of A. V. Hill (3) and his associates in England and of Meyerhof (4) in Germany. The figures here found show that, whatever proportions of fat and sugar a man is burning during rest prior to work, he continues to consume during a short period of great exertion. Sugar is therefore not the sole fuel of muscular energy. It is, however, the fuel most immediately available for muscular work and is utilized with much less distress to the subject than is fat, as Krogh and Lindhard (5) have shown. Sugar produces 5.05 calories for each liter of oxygen absorbed by respiration; from fat only 4.7 calories are obtained per liter of oxygen, 7 per cent less on the same volume of air breathed and oxygen consumed.

It has recently been shown by Levine, Gordon, and Derick (6) that in Marathon runners the sugar immediately available in the blood may be

TABLE 1

Respiratory exchange and development of power

Five members of Olympic Championship Crew. Data on volume of air breathed, oxygen consumed, CO_2 produced, respiratory quotient, oxygen deficit, total energy expenditure, external work, and gross efficiency.

	JANUARY 14—H. T. K.			JANUARY 16—B. M. S.			JANUARY 18—F. S.			FEBRUARY 28—L. G. C.			MAY 29—A. M. W.			MAY 29—H. T. K.			MAY 29—B M S.		
	No. 6 in Varsity Crew Height 6 feet 2 inches Weight, 185 pounds Pulled 36 strokes per minute on machine			No. 7 in Varsity Crew Height 6 feet 4 inches Weight, 180 pounds Rowed 30 strokes per minute on machine			No. 2 in Varsity Crew Height 5 feet 11 inches Weight, 177 pounds Rowed 33 strokes per minute on machine			No. 1 in Varsity Crew Height 6 feet 1½ inches Weight, 175 pounds Rowed 26 strokes per minute on machine			No. 3 in Varsity Crew Height 6 feet 1 inch Weight, 180 pounds Rowed 33 strokes per minute on machine			No. 6 in Varsity Crew Weight and height as on January 14 Rowed 36 strokes per minute on machine			No. 7 in Varsity Crew Weight and height as on January 16. Had missed breakfast. Test at 12:30–1:00 p.m. Rowed 40 strokes per minute on machine		
	Rest	Rowing 5 minutes	Rest 15 minutes	Rest	Rowing 5 minutes	Rest 15 minutes	Rest	Rowing 4 minutes	Rest 15 minutes	Rest	Rowing 5 minutes	Rest 15 minutes	Rest	Rowing 4 minutes	Rest 10 minutes	Rest	Rowing 4 minutes	Rest 10 minutes	Rest	Rowing 3 minutes	Rest 10 minutes
Air breathed, liters per minute...	7.5	29.00 (1)*	40.00 (a)*	9.8	33.0	44.0	8.4	32.0	31.0	9.1	26.0	29.0	12.0	49.0	44.0	13.1	51.0	38.0	11.4	53.0	60.0
		55.00 (2)	24.00 (b)		59.0	25.0		54.0	19.0		49.0	16.0		58.0	33.0		75.0	24.0		82.0	33.0
		61.00 (3)	12.00 (c)		67.0	11.0		61.0	12.0		56.0	13.0		75.0	17.0		69.0	14.0		79.0	16.0
		59.00 (4)	8.1 (d)		67.0	11.0		61.0	9.2		58.0	9.2		77.0	13.0		67.0	11.0			11.4
		64.00 (5)			70.0						51.0										
CO_2 exhaled, per cent in expired air	4.82	4.94	4.93 (a)	3.14	4.6	3.3	2.8	4.6	3.7	3.0	3.9	3.7	2.9	4.25	3.65	3.6	3.4	4.4	3.3	3.6	3.4
			5.07 (b)			2.6			3.9			3.7			3.0			3.4			3.2
			4.38 (c)			2.8			4.2			3.4			2.8			3.5			3.2
Oxygen consumed, (true) per cent from expired air	5.88	5.64	5.05 (a)	3.8	5.3	3.7	3.5	5.6	4.5	3.2	4.6	4.0	3.65	4.7	3.3	4.25	4.65	5.0	4.42	5.00	4.49
			4.57(b)			3.1			3.9			4.1			3.75			4.5			4.43
			5.00 (c)			3.5			4.9			4.3			3.8			4.4			4.53

Respiratory quotient (true)	0.820	0.875	0.977(a) 1.127(b 0.876(c)	0.83	0.86	0.90 0.84 0.80	0.80	0.81	0.83 1.00 0.86	0.94	0.86	0.91 0.90 0.78	0.794	0.904	1.096 0.800 0.737	0.857	0.732	0.88 0.75 0.80	0.75	0.72	0.76 0.72 0.71
Respiratory quotient for rowing and rest afterward			0.92			0.85			0.82			0.85			0.78			0.77			0.73
Oxygen consumed, liters per minute	0.442	1.63 (1) 3.10 (2) 3.44 (3) 3.32 (4) 3.60 (5)	2.05 (a) 1.07 (b) 0.58 (c)	0.34	1.75 3.13 3.55 3.55 3.71	1.61 0.77 0.41	0.295	1.79 3.02 3.41 3.41	1.39 0.73 0.50	0.29	1.19 2.25 2.57 2.67 2.34	1.17 0.66 0.55	0.438	2.03 2.72 3.52 3.62	1.46 1.26 0.638	0.55	2.37 3.48 3.21 3.11	1.91 1.08 0.63	0.504	2.65 4.10 3.93	2.68 1.46 0.67
Oxygen deficit repaid during rest, liters			6.46			4.00			6.56			5.53			6.45			5.10			8.07
Energy expenditure, calories per minute	2.13	21.33		1.65	18.90		1.42	21.83		1.44	15.89		2.10	22.01		2.68	20.72		2.4	29.37	
External work, calories per minute		5.4			4.5			4.95			3.90			4.95			5.4			6.0	
Efficiency, gross, per cent		25.3			23.7			22.6			24.0			22.5			26.0			20.4	

* The figures (1) to (5) in parentheses are the successive minutes of the period of rowing and are to be understood as applying to all experiments, although noted only on the first here given. Similarly, the letters (a) to (e) are the successive periods of the rest after rowing in which the expired air was collected, measured, and analyzed; (a) the first 2 minutes, (b) the next 3 minutes, (c) the next 9 minutes, or 4 minutes in some experiments, and (d) the last minute; while (e) is the last 10 minutes, or last 5 minutes in some experiments.

TABLE 2

Respiratory exchange and development of power

Oarsmen in Second Varsity and other boats. Data as in table 1

	JANUARY 14—H. C. P.			FEBRUARY 14—T. H. R.			FEBRUARY 18—A H. P		
	Crew Squad Height, 5 feet 11¼ inches Weight, 175 pounds Pulled 26 strokes per minute on machine			Crew Squad Height, 5 feet 11¼ inches Weight, 175 pounds Pulled 33 strokes per minute on machine			Second Varsity Crew, 1924 Height, 6 feet 2 inches Weight, 171 pounds Pulled 26 strokes per minute on machine		
	Rest	Rowing 5 minutes	Rest 15 minutes	Rest	Rowing 4 minutes	Rest 15 minutes	Rest	Rowing 5 minutes	Rest 15 minutes
Air breathed, liters per minute..	7.5	23.0	35.0	8.1	36.0	42.0	8.1	26.0	24.0
		53.0	15.0		68.0	27.0		43.0	14.0
		48.0	10.0		72.0	13.0		51.0	9.0
		59.0	8.1		79.0	10.4		54.0	7.0
		65.0						57.0	
CO_2 exhaled, per cent in expired air.....	4.0	4.4	4.3	4.0	3.8	3.9	3.3	4.6	4.3
			3.9			3.8			4.3
			3.8			3.3			3.6
Oxygen consumed, per cent from expired air.....	5.5	5.6	4.2	6.1	5.5	3.4	4.8	6.3	3.6
			3.9			3.9			3.7
			5.0			4.2			4.9
Respiratory quotient.....	0.74	0.79	1.02	0.66 (?)	0.70	0.98	0.67 (?)	0.73	1.18
			0.98			0.99			1.16
			0.77			0.78			0.726

Respiratory quotient for rowing and rest afterward.....			0.83			0.80			0.82
Oxygen consumed, liters per minute.....	0.41	1.29	1.45	0.49	1.98	1.67	0.39	1.64	0.88
		2.97	0.59		3.74	1.03		2.71	0.51
		2.69	0.48		3.96	0.59		3.21	0.44
		3.30			4.34			3.40	
		3.64						3.59	
Oxygen deficit repaid during rest, liters			3.29			4.95			2.31
Energy expenditure, calories per minute.....	1.94	16.49		1.29	21.77		1.82	16.18	
External work, calories per minute.....		3.9			4.95			3.9	
Efficiency, gross, per cent.....		23.6			22.7			24.1	

TABLE 3

Respiratory exchange and development of power, 150 pound crew

Data as in table 1

	JANUARY 20—N. E. F.			FEBRUARY 8—J. C. B.		
	150 pound crew Height, 6 feet Weight 155 pounds Pulled 22 strokes per minute on machine			150 pound crew Height, 5 feet, 10¾ inches Weight 154 pounds Pulled 22 strokes per minute on machine		
	Rest	Rowing 5 minutes	Rest 15 minutes	Rest	Rowing 5 minutes	Rest 15 minutes
Air breathed, liters per minute.	9.6	22.0	30.0	8.7	21.0	17.0
		32.0	15.0		46.0	16.0
		39.0	10.0		49.0	10.0
		42.0	9.2		49.0	8.7
		46.0			51.0	
CO_2 exhaled, per cent in expired air	3.0	4.3	3.8	3.1	4.6	3.6
			2.8			3.3
			3.1			
Oxygen consumed, per cent from expired air	3.5	6.2	3.8	3.9	5.2	4.4
			2.7			3.5
			4.1			4.1
Respiratory quotient	0.86	0.69	1.00	0.80	0.89	1.05
			1.04			1.03
			0.70			0.81
Respiratory quotient for rowing and rest afterward			0.76			0.91
Oxygen consumed, liters per minute	0.34	1.36	1.16	0.33	1.09	0.73
		1.98	0.41		2.39	0.55
		2.42	0.40		2.55	0.40
		2.60			2.55	
		2.85			2.65	
Oxygen deficit repaid during rest, liters			2.40			2.09
Energy expenditure, calories per minute	1.6	13.05		1.6	12.78	
External work, calories per minute		3.30			3.30	
Efficiency, gross, per cent		25.3			25.9	

consumed during a long race and that collapse in men participating in a Marathon contest is largely due to depletion of sugar. It appears probable that the same condition may occur sometimes, although rarely so intensely, in oarsmen during a four-mile race. In the test on B.M.S. on May 29 (in table 1) it was found that the respiratory quotient was only a little above 0.7 for all periods, both of rest and work. He had missed his breakfast, had eaten nothing for 18 hours, and was burning his body fat. In rowing on the machine he made a great exertion and reached the highest energy expenditure, both internal and external, of all the men examined. But his percentage efficiency in this test was below that for January 16 (in table 1) on a respiratory quotient well above 0.8.

The amount of sugar consumed by an oarsman during a four-mile race at a respiratory quotient of 0.8 may be roughly estimated at 22 grams. Sugar would then supply one-third of the carbon burned, and less than one-fifth of the energy, the greater part of the carbon and energy being drawn from fat. On a respiratory quotient of 0.9 about 55 grams of sugar would be consumed and would supply half the energy and two-thirds of the carbon. To draw the entire energy, amounting to 440 calories for a four-mile race (20 calories per minute for 22 minutes) from sugar would require 110 grams of sugar. It is probable that the more nearly the respiratory quotient approaches 1.0 just before an athletic contest, the better an athlete's "wind" and the more prolonged his endurance. It would probably not be advisable (for other reasons) to keep the respiratory quotient constantly higher than 0.9, or to increase the ration of cane sugar for athletes except quite temporarily; but it would certainly be well worth while to test in practice the effects of eating a quarter of a pound of some simple candy, such as peppermint creams, a half to three-quarters of an hour before any prolonged contest.

The sugar of the blood is glucose and is chiefly derived, not from saccharose (cane sugar), but from the starch of bread, potatoes, etc. The conversion of starch into blood sugar is however delayed by digestion, while with saccharose the utilization is rapid, too rapid in fact to use habitually, but advantageous for intense exertion.

Oxygen on credit. Among the most interesting figures obtained are those showing the "oxygen deficit" occurring during rowing, and repaid during rest after exertion. These figures represent the excess of work performed, estimated in liters of oxygen during the period of rowing, over and above the amount of oxygen that the lungs could actually absorb, or the blood transport to the muscles during exercise. The maximum amount of oxygen these oarsmen were capable of taking from the air, during an amount of work equivalent to a four-mile race, is about 3.5 liters per minute, and as a maximum during a shorter period of most intense exertion, about 4 liters a minute or a very little more. They are capable,

however, during a short period of intense exertion of developing an amount of energy during a few minutes far in excess of the oxygen simultaneously consumed. The investigations of Hill and Meyerhof, already referred to, have shown that in muscular contraction the process is anaerobic, that is, involves no oxygen but merely the breaking down of sugar into lactic acid. The consumption of oxygen occurs during the recovery process, while the muscle is being recharged preparatory to further work. The fact that more work can be done by the body than would correspond to the simultaneous consumption of oxygen, is explained by Hill as due to the accumulation of lactic acid in the muscles. This accumulation, which has later to be disposed of, partly by oxidation to CO_2, and in larger part by reconversion into sugar and glycogen, involves the continuance of a large oxygen consumption during the period of rest following exertion. It is like the financial credit of a business man at the bank; he borrows all it will allow while putting through a big piece of work, and repays later. The figures given in the tables opposite the heading "oxygen deficit" indicate therefore the total oxygen consumed during the period of rest after recovery over and above the rate of oxygen consumption during the period of rest prior to work. It is probable that in none of the men tested was the capacity to borrow oxygen extended to the full limit of their possible credit. The credit evidently was not less than 8 liters of oxygen. Assuming that this could be drawn on in two minutes, any one of these athletes would be enabled to make, during the beginning of a race or during a spurt, an energy expenditure at least double the amount corresponding to the oxygen simultaneously consumed.

Variations of energy expenditure. The basal energy expenditure for a man of the height and weight of a varsity oarsman is, according to the DuBois (7) standard, 1.4 to 1.5 calories per minute; that of a man on the 150 pound crew slightly less. During the period of preliminary rest, the data here reported show that in a few cases the basal figure was approximated, but that in others it was considerably exceeded. During work the total energy expended per minute was from 15 to 20 times this basal value, or about 10 times the value during sitting rest. The figures for the number of calories liberated per minute during rowing on the machine were obtained by adding the amount of the oxygen deficit, which was made good during rest after exercise, to the total oxygen consumed during the period of rowing and dividing by the number of minutes during which the man rowed. The figures so obtained, multiplied by the value for the calories liberated per liter of oxygen consumed, give the figures in the tables.

The figures for the external work were obtained from the rowing machine in the manner already described. They were calculated initially in foot pounds per minute, but for purposes of comparison have been here set down in their heat equivalents as calories per minute.

Percentage efficiency. The percentage which the external work on the rowing machine (that measured plus 25 per cent) bore to the total energy development, as estimated from the oxygen consumed and the oxygen deficit, is expressed in the last line of figures in table 1. The efficiency is here seen to lie between 20 and 26 per cent, figures which are exceeded by few engines motivated by the burning of carbonaceous fuel. The data obtained on oarsmen who were on the crew squad but not on the varsity eight, as shown in table 2, indicate practically equal power and mechanical efficiency. Data from two men of lighter build on the 150 pound crew, given in table 3, show a high percentage efficiency, but indicate that their maximum power was considerably below that of the heavier men.

TABLE 4

Power developed in rowing various speeds and distances

From data obtained by three distinct methods. Power expressed in calories, i.e., as heat equivalents of work.

General conditions:				
Number of strokes per minute in boat or on rowing machine	30	33	36	40
Length of time such exertion can be maintained, i.e., duration of boat races, minutes and seconds	22-0	16-0	10-20	6-15
Speed of boat, minutes and seconds per mile	5-30	5-20	5-10	5-0
External work of an oarsman, expressed in calories, as determined:				
(1) From drawbar pull and speed	4.78	5.06	5.47	6.00
(2) From rowing machine	4.50	4.95	5.40	6.00
(3) From oxygen consumed (assuming efficiency of 25 per cent up to 36 strokes, and 20 per cent at 40 strokes per minute)	4.72	5.48	5.26	5.85

Energy expenditure at racing speeds. We are now in position to estimate by three distinct methods the external work done by an oarsman during a race: *a*, by the draw bar pull and speed when a boat is towed; *b*, by the work measured on the rowing machine in the laboratory; and *c*, by determination of the amount of oxygen consumed. The data from these three methods are summarized and compared in table 4. In order to make the comparison several assumptions are necessary, which we must recognize are only approximately correct; and even so there are some discrepancies. On the whole, however, the agreement of the various measurements with each other and with the records of the speed established in races for various distances is quite satisfactory.

The records of races are the truest measurements; for the time of any

race, the distance covered, the number of strokes per minute and the energy expended represent a maximum of human power for each set of conditions more exact than any cold-blooded laboratory determination. Somewhat arbitrarily, assuming no current in the water and no windage, we have set the conditions, in the first column of figures in table 4, for a four-mile race at 22 minutes, or 5 minutes and 30 seconds per mile, and at thirty strokes per minute. The external work done by an oarsman has been computed by each of the three methods, transformed into its heat equivalent and stated in calories per minute. The resulting figures are 4.78, 4.50 and 4.72; a remarkably close agreement. In the figures of columns 2 and 3 of table 4, the number of minutes that the exertions can be maintained are given as approximately those for races of two and three miles respectively. The data for the draw bar pull and the rowing machine agree remarkably well; those for the oxygen determination less well, however. In fact the data happen to indicate an impossibility, namely, that less oxygen is consumed and less energy expended at a stroke of 36 than at a stroke of 33 per minute. The discrepancy is in large part due to the fact that both the available determinations at 36 strokes, given in table 1, were made on H.T.K., number 6 in the boat, and an oarsman—we might almost say a machine—of extraordinary efficiency.

For a race of one and a quarter miles at a speed of a mile in 5 minutes and a stroke of 40 per minute, the power per man expressed in calories per minute, by the three methods, comes to 6.00, 6.00 and 5.85; an agreement so close as to involve a certain amount of good luck. The figures fix with a high degree of certainty the maximum of human power for an exertion of this duration. Given in other units 6.0 calories per minute is nearly 0.57 horse power, or 18,769 foot pounds, or 2580 kilogram meters per minute. If the efficiency is 20 per cent the total energy expended would be 5 times each of these figures, or 30 calories per minute, and would require the oarsman to consume 4 liters of oxygen a minute, which appears to be all that his lungs, blood and heart can absorb and transport to his muscles; and also to incur a debt for 2 more liters of oxygen each minute of the exertion—a debt to be repaid during the recovery period of deep breathing after the race. A total expenditure of 29.5 calories per minute is about 20 times the basal rate at rest in bed before breakfast.

Food and fuel of athletes. There is a fourth method for determining energy expenditure, namely, by the calorific value of the diet; but it is not applicable to so short an exertion as that of rowing. None the less the nature of the fuel, which is consumed in an oarsman's muscles and propels the boat, and the frequency with which the vital engine should be stoked, are topics that would repay thorough scientific study in the light of modern knowledge of nutrition. Most of the ancient superstitions, such as the diet of raw beef for Roman gladiators and English prize fighters, which still

influenced the training table down to quite recent times, have now disappeared; but not all are gone, nor have they in all cases been replaced by well-founded conceptions. The data here reported indicate that at least two-thirds of the energy expended by an athlete now is derived from fat. There is a very significant saying that "in the living body fat burns only in a flame of sugar"; meaning that with deficiency of sugar the oxidation of fat also is incomplete. It would therefore probably be distinctly helpful to "wind" and to the prevention of overtraining if athletes were fed so that their respiratory quotients were kept constantly well up to 0.85 or even 0.9. In addition, as already suggested, it would probably be advantageous to further raise the respiratory quotient and to provide ample sugar in the blood and tissues, a half or three-quarters of an hour before any prolonged contest. Sugar is the best quick fuel for intense exertion.

CONCLUSIONS

The energy expended in rowing an 8-oared racing shell for the various distances and speeds occurring in intercollegiate and other contests has been here determined by three methods: 1, By the draw bar pull and speed when the boat and crew are towed by a motor boat; 2, by means of a rowing machine, an efficient ergometer, in the laboratory; and 3, by determinations of the volume of oxygen consumed from the air by the oarsman's breathing. The results by these diverse methods agree satisfactorily. They indicate that the maximal power exerted is from 0.45 to 0.57 horse power per man, or expressed in the heat equivalents, 4.8 to 6.0 calories per minute (see table 4), with a total energy expenditure of 19 to 30 calories per minute, or 13 to 20 times the basal rate. The power expressed by the smaller of each of these pairs of figures is that maintained, and is therefore approximately the maximum that a man can maintain, for twenty-two minutes during a four-mile race; while the higher figures are applicable to the more intense exertion and greater speed, which are also maximal, for about six minutes in races of about one and one-third miles or 2000 meters. The corresponding figures for the volume of oxygen consumed per minute are 3.5 and 4 liters; the latter figure is the limit of the transporting capacity of the lungs, blood and heart. An oarsman exerts a power, which exceeds by 30 to 60 per cent that afforded by the oxygen simultaneously absorbed; he thus draws heavily on his credit, and incurs oxygen deficits of 4 to 8 liters or more; and these deficits are repaid by the high rate of oxygen absorption for a time after the work is ended.

The most significant result of these observations is the conclusive evidence which they afford that in whatever proportion fat and sugar are being burned during rest just before the exercise, they are burned in nearly the same proportion to produce the energy for doing work or for the recovery process in the muscles. A man can even make an intense exer-

tion, although rather disadvantageously, on a combustion almost entirely of fat from his own body. The advantage of increasing the proportion of sugar is pointed out.

In contrast to the effects of great exertion on untrained men, there was in the members of this crew only a slight overbreathing, or sometimes practically none at all, with a correspondingly slight blowing off of CO_2 during work or afterward. Apparently some of the phenomena, especially the blowing off of CO_2 and the high respiratory quotient during and immediately after intense exertion, which are commonly explained as due to the development of an "acidosis," and which might be interpreted as strongly supporting the Hill-Meyerhof conception of muscular contraction, are due to the stimulation of the respiratory nervous regulation by the incomplete oxygenation of the arterial blood during great exertion, rather than to displacement of carbonic acid from the blood bicarbonates by lactic acid.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XV. Pseudaffective Medulliadrenal[1] Secretion

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The bodily reactions attending great emotional excitement are elemental. In many respects they are common to man and to lower animals of the vertebrate series. They involve changes which may be regarded as instinctive adjustments to critical situations, and as directed toward self preservation. They may dominate behavior. They may thoroughly disturb beneficent functions, such as secretion and digestion. A method which will permit the securing of further insight into emotional reactions is therefore important.

The student of emotional reactions is confronted by almost insuperable difficulties. In order to study intimately the changes associated with great excitement it is necessary to examine as exactly as possible the functioning of various organs or systems of organs in the body. This often cannot be done at all unless the animal is subjected to anesthesia. So far as emotion is concerned, anesthesia, of course, promptly obliterates the very responses which are the object of study. But even under circumstances in which anesthesia would not be required there is frequently trouble in establishing consistently the emotional states which we wish to study. The natural antagonism between two laboratory animals, the dog and the cat, has been used to induce an emotional reaction. The excite-

[1] The functional separability of the adrenal cortex and the adrenal medulla emphasizes the importance of using terms to apply to each portion of the adrenal body that will distinguish between them. One may use the expression "medulla of the adrenal gland" or the "adrenal medulla" to discriminate that portion from the gland as a whole. The latter expression is clear, but unfortunately does not yield an adjective form. As a means of indicating briefly and distinctly the part of the adrenal structure which is under consideration, I propose, at the suggestion of Prof. E. K. Rand of the Department of Latin, Harvard University, that the adrenal medulla be referred to as the "medulliadrenal gland" and that the cortex be referred to as the "corticadrenal gland."

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ment produced by confronting these two animals is likely, however, to be temporary, for a disturbance which at first may be marked becomes gradually less pronounced as the animals grow accustomed to each other. Again, during the brief period of the reaction opportunity to make careful observations can hardly be obtained, and furthermore such measures as may be taken for studying the response may interfere disastrously with the response itself.

The possibility of obtaining deeper insight into the physiological aspects of emotional excitement is suggested by previous proof that central mechanisms for emotional reactions can be artificially activated in the decerebrate animal, and also by evidence that the thalamic region plays an important rôle as a center for emotional expression. In 1904 Woodworth and Sherrington (1) described certain phenomena which could be called forth from the background of decerebrate rigidity. These phenomena resembled the expression of emotional states, and were designated "pseudaffective reflexes." In Sherrington's words, "The truncation of the brain of the mammal at the mesencephalon annihilates the neural mechanism to which the affective psychosis is adjunct. But it leaves fairly intact the reflex motor machinery whose concurrent action is habitually taken as an outward expression of an inward feeling." These quasi-emotional reactions, induced by stimulating an afferent nerve, included movements of the limbs as in progression, turning of the head and neck toward the points stimulated, opening the mouth, retraction of the lips and tongue, movement of the vibrissae, snapping of the jaw, lowering of the head, opening of the eye lids, dilatation of the pupils, snarling or plaintive vocalization and a transient increase of arterial blood pressure. The reactions appeared not only in combination but sometimes singly or in small combinations. Bazett and Penfield (2) have reported that in the "chronic" decerebrate cat the pseudaffective phenomena are more developed after the first few days than immediately after operation, and that in addition to the activities listed by Sherrington they noted lashing of the tail, protective movements of the fore limbs, kicking and biting and in some cases growling.

Hughlings Jackson (3) and Head (4) have pointed out that the nervous system is organized in a neural hierarchy such that primitive reactions, which might otherwise disturb the more discriminative responses of higher levels, are by these repressed. The rigidity of the decerebrate preparation may thus be explained as due to the "release" of a neuro-muscular apparatus that is normally kept under dominance of the cerebrum. That the pseudaffective phenomena described above can be made to intrude upon the rigidity indicates that a neural mechanism for these phenomena is present in the nervous system below the pons. That they do not manifest themselves unless evoked by special excitation indicates that the mech-

anism involved, though released from cerebral control, is itself suppressed by or subordinated to the apparatus that is active in maintaining rigidity.

The thalamic region comprises a group of centers which, in the lower vertebrate, lacking a cerebral cortex, serve the primitive functions for maintaining existence. These centers, though normally under the inhibitory control of the cortex, are capable of energetic response when conditions demand urgent and impulsive action. If the cortical government is set aside the subordinate activities become prominent. Goltz's (5) hemisphereless dog and Barenne's (6) cats deprived of the neopallium are cases in point. Even to conditions which are ordinarily agreeable the animals reacted by signs of rage. The dog barked, snapped and struggled vigorously when taken by his keeper to be fed, and the cats when lifted up growled and hissed—i.e., their reactions were part of the well-known complex for resistance or defense. In human cases, likewise, as Head and Holmes (7) have noted, such damage to the brain as isolates the thalamus from the cortex has as its most remarkable feature "an excessive response to affective stimuli." Removal of the cortex in an acute experiment might, therefore, permit the activity of these lower centers to appear, and to prevail over the rigidity which usually masks them when the mid-brain is transected. Typical and stereotyped behavior should then occur, such as may be natural in attack or defence. Indeed, just those mechanisms might be brought into action that operate during the instinctive efforts of animals to escape from danger or to fight for existence. In that case the well-known visceral accompaniments of these instinctive acts might reasonably be expected to be present.

The foregoing ideas suggest the possibility of obtaining a preparation in which the basal ganglia, freed from the dominance of the cortex, might function in a manner which would exhibit many of the physiological phenomena attending intense emotional disturbance. Destruction of the cortex would eliminate an essential factor for sensation; and therefore the use of an anesthetic, which would disturb not only nervous but probably other functions, could be dispensed with. The absence of the higher inhibiting centers would permit a *prolonged* exhibition of the quasi-emotional phenomena. These phenomena could be studied experimentally during the period without any chance of interrupting the relatively simple automatic activities. The methods used in the study of such a preparation and the results obtained from it might then be applied to intact animals under more natural conditions to ascertain the degree to which the quasi-emotional states match the conditions of real excitement. It was with these purposes in mind that the present investigation was undertaken.

Methods. In order to bring about a destruction of the cortex without destruction of the basal ganglia we have made use of the Sherrington decerebrator, and also we have approached the brain by way of the orbits. When the decerebrator was employed it was necessary to slant the knife

well forward so as to remove chiefly the dorsal cortex and the frontal lobes. As a rule we have obtained more consistent results through the orbital approach. After both carotid arteries have been tied a pointed stylet, dulled at the end, is thrust through the upper inner quadrant of the right orbit and directed toward the posterior portion of the zygomatic arch of the left side. When the stylet has reached nearly to the left cranial wall of that region it is moved up and down in a plane slanting downwards and outwards. The movement is continued as the stylet is withdrawn, thus effectively destroying the left frontal lobe and left lateral cortex. The instrument is then similarly introduced into the left orbit, and the right frontal lobe and right lateral cortex are likewise destroyed. A *post-mortem* examination of the brain was made in practically all cases. The plane of section usually passed about midway through the orbital lobe, thence obliquely across to the opposite hemisphere, cutting through the genu of the corpus callosum and ablating 2 to 4 mm. of the thalamus, and emerged a few millimeters posterior to the Sylvian fissure. The region of the cat's cerebral cortex, found by Barenne (8) to subserve sensation, including pain, was thus quite disconnected from the rest of the brain. A wedge of the occipital cortex does not suffer direct injury. Usually there is no considerable amount of hemorrhage.

In some instances we wished to know to what degree the effects produced by the decortication just described could be obtained under decerebration. We found that the skull about 1 cm. dorsal to the posterior end of the zygomatic arch is sufficiently thin to permit the stylet to be thrust through it readily. By appropriate movement of the instrument after it has penetrated the brain case, the active pseudaffective state can be quickly changed to a state of decerebrate rigidity.

In our experience all the operative procedures should be performed with the utmost rapidity in order that the period of anesthesia may be as short as possible. When the period of anesthesia is prolonged many of the phenomena to be described later do not occur. On the other hand, if the operation is begun as soon as ether has rendered the animal insentient, and if the ether is removed as soon as the swift cerebral ablation has been accomplished, there is usually a very good showing of the quasi-emotional phenomena.

The phenomenon related to the pseudaffective state that we are reporting in this paper is secretion from the adrenal medulla. In testing the presence of increased and varying medulliadrenal secretion we have employed the denervated heart, the use of which for that purpose has been justified in previous papers (9). Fasting animals (cats) were selected, to make sure that the hepatic factor would play only a temporary and negligibly minor rôle in causing cardiac acceleration (10). Thus there was no delay due to abdominal operation. As an indication of the speed

with which the entire preparation can be made ready, when two skilled persons coöperate, we cite the following typical protocol:

October 15, 1924. Cat, male; 3.6 kilos.

10:27. Ether started
10:31. Right carotid artery tied and right vagus nerve cut
10:31½. Left carotid artery tied and left vagus nerve cut
10:32. Tracheal cannula inserted
10:33. Decortication *via* orbits. Ether stopped (used thereafter only to check movement during operation)
10:35½. Thorax opened and right stellate ganglion excised
10:37. Thorax opened and left stellate ganglion excised
10:39. Thorax closed
10:42. Right femoral artery exposed and cannula inserted
10:42½. Rectal thermometer introduced
10:43. First kymographic record taken

In this instance the period from starting anesthesia until a blood-pressure tracing was obtained was 16 minutes. Our shortest record was 14 minutes, our longest 22 minutes. Usually the procedures did not require more than 17 minutes.

As in previous cases in which the denervated heart has been employed to indicate medulliadrenal secretion, the temperature of the animal, as shown by a thermometer in the rectum, was maintained as nearly as possible within a variation of one degree Centigrade from the initial temperature.

Results. When the operation is performed as described above there appears quite spontaneously a group of remarkable activities such as are usually associated with emotional excitement—a sort of sham rage. A complete list of these pseudaffective phenomena which we have observed is as follows: lashing of the tail; vigorous arching of the trunk, and thrusting and jerking of the limbs in the thongs which hold them to the animal board, combined with a display of claws in the fore feet and clawing actions, often persistent; rapid head movements from side to side with attempts to bite; very rapid panting respiration, developing usually within 10 or 15 minutes after decortication and reaching a state precisely similar to that seen after great exertion or on being overheated (the mouth is held open, the tongue is moved rapidly to and fro, the nares widen synchronously with each breath; the breathing is very superficial and in eight cases in which it was recorded reached the remarkably high range of from 288 to 304 breaths per minute (e.g., see fig. 1)). These activities occur spontaneously, but in "fits" or periods, lasting from a few seconds to several minutes. During the intermediate quiet stages a "fit" may be evoked by slight handling, touching the paws or jarring the table. In the quiet periods some evidence of rigidity was usually found to be present, or could be obtained by bending the fore legs; when rigidity was marked, as in a few experi-

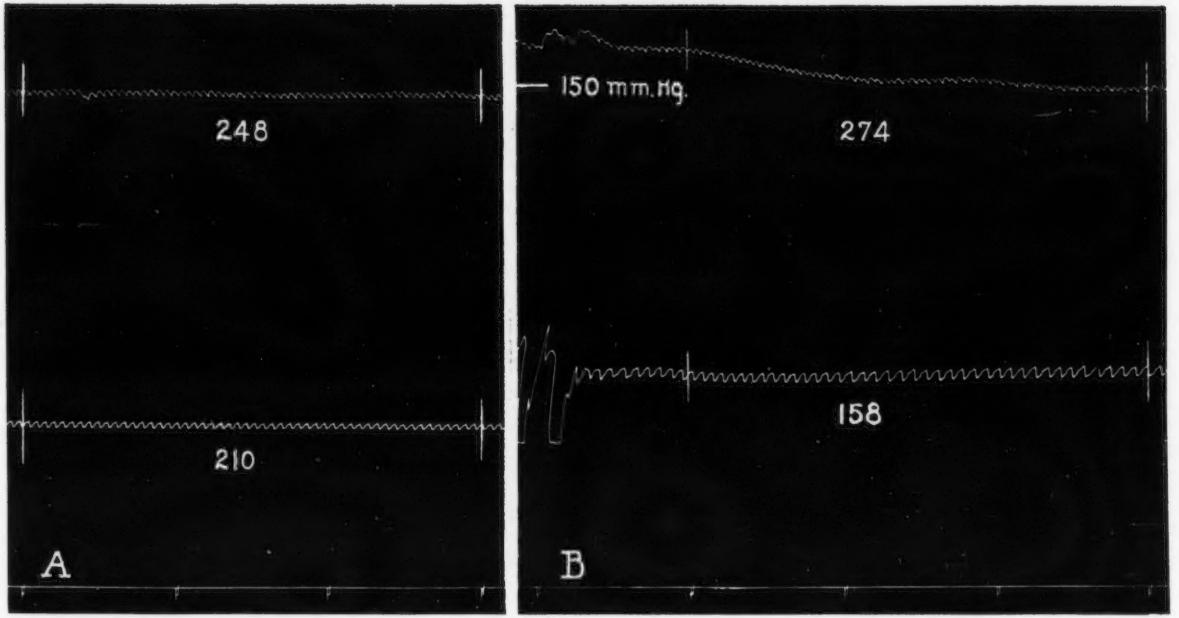

Fig. 1. Experiment of October 29. Record of blood pressure, cardiac and respiratory rates in the pseudaffective condition (original size). Base line registers zero blood pressure and time in 5-second intervals. *A*, 11:30, during quiet (heart rate, 248 beats per minute; respiration, 210 per minute); *B*, 11:32, immediately after activity (heart rate, 274 per minute; respiration, 158 per minute). Tail hairs erect, hind paws wet with sweat.

ments, and as happened not uncommonly in the late stages of experiments, the pseudaffective features became correspondingly less pronounced.[3]

Besides the foregoing changes which involved skeletal muscle there were typical and more permanent visceral effects. Erection of the tail hairs,

TABLE 1

TIME	RECTAL TEMPERATURE	HEART RATE	BLOOD PRESSURE	RESPIRATION	NOTES
10:43	36.5	204	190		Animal quiet
10:50		188	160	80	Panting started. Hind toe pads moist, front pads dry
10:55		184	150	180	Clawing, tail slightly rough; hind toes wet; mouth open and tongue moved as in panting
10:59	37.5	180	150	216	Quiet
11:00		196	186	180*	Spontaneous activity
11:09		180	156	228	Quiet
11:10		200	170	140*	Activity—jerking and thrusting
11:15	37.2	180	160	288	Quiet: other conditions as at 10:55
11:20	37.2	178	140	276	Quiet
11:21		202	164	48*	Spontaneous jerking, raising of head, etc.
11:30	36.4	184	150	240	Quiet. Tail rough; hind toes wet; panting respiration
11:37					
11:51					Very light etherization to abolish reflex activity while both *adrenals were removed*
11:54	37.8	159	76	156	Quiet
12:0		156	90	86	Quiet
12:05		158	108	34	Toe pads not perspiring; tail slightly rough
12:20	38.3	160	108	26	No change of condition
12:30		156–158–154	100–110	25	Stimulation of exposed right femoral nerve for 30 sec.; respiration deepened
12:35		158–160–160	110–156–130–100		Stimulation of left brachial nerve for 30 sec.; great activity
12:40		158	90		Animal killed by clamping trachea with no change in heart rate during the asphyxia
12:50		158			

* The slower respiratory rate brought on by activity was quite characteristic (see also fig. 1).

[3] Recent interest in the double innervation of skeletal muscle, and in the rôle of sympathetic nerves in the maintenance of its tone, makes appropriate the report that in many of our cases marked rigidity of the fore limbs was noted, although the stellate ganglia through which the sympathetic impulses to the fore limbs pass, had been completely removed.

which recurred again and again after they were smoothed down; sweating of the toe pads, in some instances so marked as to wet the hairs between the pads (this phenomenon was absent from the fore paws when the stellate ganglia were removed, and sometimes from the paw of the hind limb in which the femoral artery was closed); dilatation of the pupil so that during activity it might be three times as wide as during a preceding period of quiet (since the stellate ganglia had been removed the effect was probably largely due to inhibition of pupillo-constrictor impulses); micturition; contraction of the rectum, with expulsion of the rectal thermometer, and sometimes defecation; and a high blood pressure ranging from 146 to 210

TABLE 2

Changes of rate of the denervated heart, and maximal blood pressures, observed in the pseudaffective state when the adrenal glands were intact

DATE	AT START OF EXPERIMENT	HEART RATE PER MINUTE			MAXIMAL BLOOD PRESSURE
		Minimal during quiet	Maximal during activity	At end of experiment	
					mm. Hg.
September 25, 1924	224	220	232	*	168
September 27	274	273	308	*	210
September 30	212	204	256	*	146
October 3	256	255	276	228	180
October 3	260	212	230	*	168
October 14	234	228	248	204	172
October 15	204	178	202	184	190
October 22	256	242	270	212	190
October 23	196	188	256	176	204
October 24	208	182	212	184	186
October 25	211	184	240	191	160
October 27	268	232	248	212	194
October 29	254	248	274	204	160
October 31	248	222	256	212	206
November 1	274	264	272	212	195

* *Experiment interrupted.*

mm. of Hg. Like the pseudaffective reflex phenomena described by Woodworth and Sherrington, these spontaneous reactions did not always appear in complete combination; occasionally several of them were absent either wholly or during a part of the experiment. In all cases we concluded the experiment by killing the animal; the preparations were usually studied from 1.5 to 3 hours.

Some of the points mentioned in the foregoing list, and also the evidence that there is increased secretion from the adrenal medulla in company with the other indications of sympathetic activity, will now be reported in detail.

In table 1 are presented the main facts of body temperature, heart rate, blood pressure and respiration at various times after the preparation had been made. The experiment was that of October 15, the operative procedures of which were detailed above (see p. 287). In table 2 are given the variations of heart rate and also maximal heights of blood pressure in 15 of our 19 experiments. The remaining 4 experiments were done by means of the Sherrington decerebrator, and because of accident or hemorrhage were unsatisfactory. Examination of table 1 brings out the fact that the rate of the denervated heart when the adrenal glands were present was well above the average rate which prevailed after they had been removed. In this case only a moderate instance of the general rule is illustrated. In previous studies in this laboratory the average rate of the denervated heart in a series of 20 fasting animals from which the adrenals had been removed was 156 beats per minute—a rate closely approximating that observed in the experiment detailed in table 1 after both adrenals had been extirpated. Comparison of this figure with the figures in table 2 reveals immediately a remarkable difference. The average minimal rate there reported during quiet was 222 beats per minute—66 beats above 156.

The rate of the denervated heart is usually high shortly after the decortication, it is likely to be lower thereafter during quiet periods, and it is seen to rise sharply during any exhibition of vigorous spontaneous activity (see tables 1 and 2 and also fig. 1). In the 15 cases in table 2 the average rate shortly after decortication was 239 beats per minute; it fell during quiet to 222, and rose during activity to 252 beats. The extraordinary rate of 308 beats per minute was recorded as the maximal rate during activity in one instance.[4] The increase during activity is not seen if the hepatic nerves are cut and the adrenal glands are removed, or is slight if the adrenal glands are removed from fasting animals (see table 1, 12:35, when marked activity and a great rise of blood pressure were induced by stimulation of a brachial nerve). Since these high rates occur while the adrenal glands are present and active, and are replaced by rates in the neighborhood of 156 beats per minute after the adrenal glands have been extirpated, we draw the inference that the adrenal medulla participates

[4] Tulgan (This Journal, 1924, lxix, 441) has declared that after bilateral vagotomy the heart rate is at its physiological maximum, and can not be increased by stimulation of a sensory nerve or by injection of adrenalin. In the figures he publishes the maximal rate for the cat is set down at 210 beats per minute. As shown in figure 1 and in table 2, this is not a "physiological maximum" in the sense that the heart is unable to beat faster than 210; it may beat very much faster than that. Furthermore, Cannon and Rapport (This Journal, 1921, lviii, 330) have recorded rates of approximately 260 beats per minute induced by injections of adrenalin. Does the denervated heart differ from the heart still innervated by the sympathetic (as was the case in Tulgan's experiments)? Does the nature of the anesthetic restrict the action of adrenalin?

in the general widespread discharge of sympathetic impulses in the pseudaffective state, and pours adrenin into the blood stream to such a degree that the denervated heart is driven for a considerable period, often two hours or more, to maintain a rapid beat. As shown in table 2, the rate toward the end of the experiment is likely to be considerably less than at the beginning. This change does not appear to be due to a diminution of the quasi-emotional activity; it is possibly the consequence of exhaustion of the adrenal medulla, but we have not determined that point.

As table 2 reveals, the maximal blood pressure is high in these cases. Rarely does the normal blood pressure of the cat under ether anesthesia range above 150 mm. Hg (11). In all but one of the cases listed in table 2 the pressure went well above that figure. One might suppose that the high blood pressure might be due to increased intracranial pressure resulting from the operation. We have observed, however, blood pressure as high as 190 mm. Hg in an animal in which the pseudaffective state was brought on by a Sherrington decerebration. The opened cranium in this case would not permit the development of a high cerebral pressure. Furthermore, the absence of any considerable hemorrhage in cases in which orbital decortication was employed would hardly warrant the supposition that a considerable intracranial pressure developed.

It was noteworthy that both the blood pressure and the rate of the denervated heart were highest in animals in which the pseudaffective phenomena were most prominent. If the decortication failed to produce the physiological signs of emotional disturbance, but rather the indications of decerebrate rigidity, the heart rate was invariably lower. Or if by further sectioning at the mid-brain the preparation was changed from the quasi-emotional state to a state of rigidity, the denervated heart beat more slowly. The average minimal rate in nine cases when the condition of rigidity was prominent was 198 beats per minute, and in six cases in which activity was replaced by rigidity after further section through the mid-brain the average rate quickly fell from 234 to 210 beats per minute. The rates in the rigid state, as will be noted, are higher than the rate seen after removal of both adrenal glands (156 beats per minute) but not nearly so high as during the active periods of a quasi-emotional exhibition (252 beats per minute). It is clear, therefore, that although medulliadrenal secretion is stimulated by the experimental circumstances which prevail in decerebrate rigidity it is stimulated still more during the pseudaffective responses.

Discussion. The results presented in the foregoing paragraphs bring support to earlier evidence that there is a true emotional secretion from the adrenal medulla. This evidence, first reported by Cannon and de la Paz (12) in 1911, was confirmed the next year by Elliott (13), who found that cats excited by new experiences in the laboratory had a smaller adrenin

content in the adrenal medulla than did quiet animals, that if the splanchnic nerves had been cut previously on one side the adrenal medulla on that side had a larger load of adrenin than did the other gland after the animals had been excited by morphia or β-tetrahydronaphthylamine, and that if the superior cervical ganglion were removed a paradoxical dilatation of the pupil occurred when the animal became angry, a reaction which failed after both adrenal glands were excised. The last of these three lines of evidence was strengthened by similar observations by Kellaway (14). Stewart and Rogoff (15) were unable to note the effects seen by Kellaway when they removed one adrenal gland and denervated the other, and they found no reduction of the adrenin content of the medulla in animals subjected to excitement. Our present observations are in harmony with the testimony of Elliott and of Kellaway and not with that submitted by Stewart and Rogoff.

A noteworthy feature of our results is the markedly faster rate of the denervated heart which appeared whenever the animal changed from quiet to aggressive activity, and the absence of that effect if the adrenal glands had previously been removed (see table 1). It would appear from the observations of Hartman, Waite and Powell (16) that any considerable muscular movement which an animal engages in is associated with increased medulliadrenal secretion. Unpublished observations made in this laboratory may be given a similar interpretation. Our results indicate that even when in an emotional state the adrenal medulla is hyperactive, the putting forth of vigorous muscular effort is associated with a still greater activity and a larger outpouring of adrenin—causing an increase of heart rate, on the average, from 222 to 252 beats per minute. These facts suggest that the somatic neuromuscular functions more or less under voluntary control, and the visceral or non-voluntary functions, that are brought into action simultaneously during excitement, are affected to a similar degree. The interesting question is raised whether inhibition of the somatic expressions of emotional disturbance may not be accompanied by a correspondingly smaller expression of the visceral functions. The control of one's behavior when there is danger that expression may be excessive may have more than superficial effects.

The facts reported in the foregoing paragraphs are merely the first results of a series of studies which we are making on the pseudaffective preparation. It is hoped that the methods and results established by study of this preparation can be tested under more natural conditions in intact animals and in man.

SUMMARY

A preparation is described in which many of the physiological accompaniments of great emotional excitement can be produced in an acute experiment, without anesthesia, and thus can be readily studied for hours.

The denervated heart in this preparation has rates averaging (in 15 experiments) 222 beats per minute during quiet, and 252 beats per minute during activity. If the adrenal glands are removed the rate of the denervated heart averages 156 beats per minute, and is not increased to a noteworthy degree (not more than 4 to 8 beats per minute) during activity. We draw the inference that associated with the physiological signs of emotional excitement in the pseudaffective state there is greatly increased secretion of adrenin from the adrenal medulla.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XVI. The Rôle of the Adrenal Medulla in Pseudaffective Hyperglycemia

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In 1911 Cannon, Shohl and Wright reported observations on the experimental production of glycosuria in emotional states (1). This emotional glycosuria, noted in cats, has since been seen in rabbits, dogs and man (2). In three instances studied by the above-mentioned investigators emotional disturbances failed to induce glycosuria after removal of the adrenal glands. The few cases studied, with the performance in them of a considerable operation which might have interfered with the reaction of the animals, did not offer strong evidence for adrenal involvement in the production of emotional glycosuria. Further examination of this point is desirable. In the present investigation we have not returned to former methods but have made use of the method of establishing a quasi-emotional state recently described by Cannon and Britton (3). This pseudaffective state presents many of the physiological features of true emotional excitement, and permits various conditions to be tested in acute experiments.

Methods. For the experiments which we have performed well-nourished cats were used. They were anesthetized with ether, both carotid arteries were tied, and a cannula was introduced into the trachea to permit artificial respiration if necessary. A short wide cannula, with a capacity of about 2 cc. but calibrated for 1 cc., was fastened into the left carotid artery low in the neck for collecting blood samples. A clip separated it from the blood stream. Another cannula was tied into the femoral artery for recording blood pressure. All these operative procedures were finished in the shortest possible time in order to minimize the duration of ether anesthesia.

As soon as the foregoing preparations had been made the animal was decerebrated by the Sherrington method, or, more commonly, decorticated by way of the orbits in the manner described by Cannon and Britton. Thereafter ether was no longer given.

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In collecting blood samples a small uniform amount of sodium oxalate was first dusted into the carotid cannula and then the clip was opened and the blood allowed to rise above the 1 cc. mark. A carefully cleaned 1 cc. pipette was then used to stir together the oxalate and the blood, whereupon the blood was drawn into the pipette, lowered to the 1 cc. mark, and after the tip of the pipette was cleaned, run into the diluting fluid. The Folin-Wu method of analysis was followed (4). After each sample

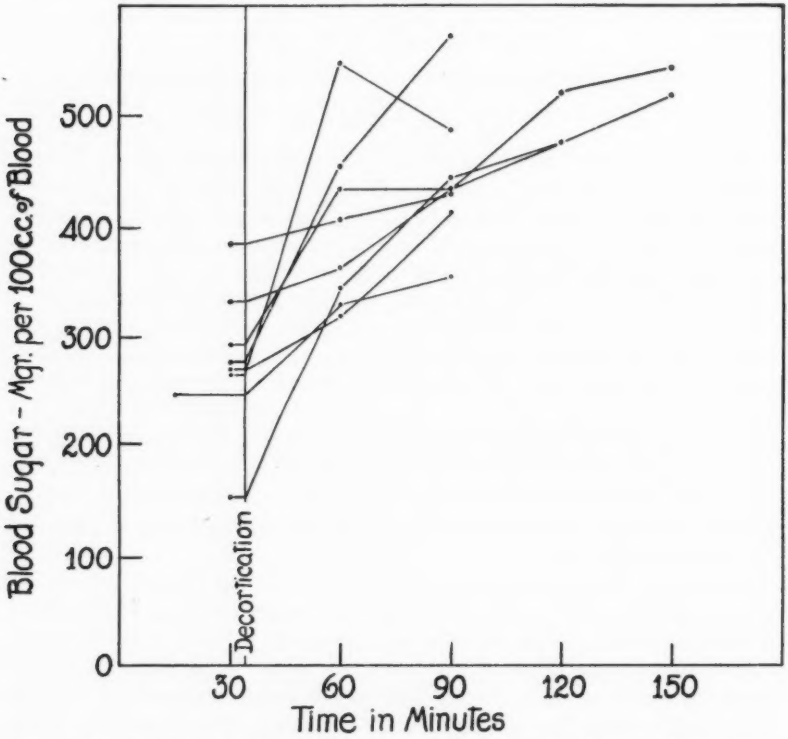

Fig. 1. Blood sugar in decorticate animals manifesting pseudaffective phenomena

was taken the carotid cannula was thoroughly cleaned. A blood sample was taken immediately before decortication, and thereafter at approximately half-hour intervals, so long as the preparation remained in good condition, i.e., with a blood pressure above 75 mm. Hg. Usually this period was about two hours and a half, though sometimes it lasted four or five hours.

Careful notes were kept of the state of the animal, and the presence or

absence of pseudaffective phenomena and rigidity. Precautions were taken to maintain the temperature of the animal at a fairly constant level throughout the experiment. Post-mortem examination of the brain was made in nearly all cases. Since we desired to know particularly whether or not the pituitary body had been disturbed we made special examination of the conditions in the pituitary region.

RESULTS. *Hyperglycemia in the pseudaffective state.* In table 1 and in figure 1 are shown in milligrams per 100 cc. the sugar in the blood as found at different stages during the experimental procedure. The first sample, taken shortly after the animal had had the excitement and asphyxia of rapid etherization and the disturbance of operation, showed a sugar level well above the normal. In the eight cases of table 1 this preliminary sample had an average sugar content of 278 mgm. per 100 cc.

TABLE 1

Blood sugar in animals decerebrated and manifesting pseudaffective phenomena

DATE	BLOOD SUGAR (MGM. PER 100 CC. OF BLOOD)					REMARKS
	Before decortication	After decortication (approximately half-hour stages)				
1924						
March 5	388	408	430			Animal active, sweat visible on paws
March 8	270	320	412			Tail hairs standing
March 14	246	330	357			Tail bushy, respiration rapid, active
March 27	276	454	571			Pseudaffective phenomena, considerable rigidity
April 3	155	347	444	476	519	Panting, clawing, wet paws
April 5	266	547	487			Jerking, bushy tail, claws out
May 15	333	363	434	520	541	Tail wagging, growing rigidity
May 27	292	434	434	476		Paws wet, clawing, very active

After decerebration the blood sugar invariably rose further. At the end of the first half-hour the average figure had risen from 278 mgm. to 400 mgm., and at the end of an hour the average was 446 mgm.,—an increase shared by all the cases except two (Apr. 5 and May 27). In three instances the observations were continued beyond the first hour after decerebration, and it is noteworthy that in all three the sugar percentage continued to rise.

In the last column of table 1 are set down the more prominent of the pseudaffective phenomena that were seen in the various cases. In all of them combinations of typical reactions were seen, revealing activity of the sympathetic system, but the combinations were not always alike, and the most outstanding evidences of sympathetic activity were not always the same. The striking fact, however, is that, despite these

variations in the superficial signs, the glycemic percentage always rose, and in five of the eight cases ran above 0.45 per cent.

Contrasted with the results shown in table 1 are the results shown in table 2 (represented graphically in fig. 2), that were obtained from decorticate animals which did not manifest any pseudaffective activity. Associated with absence of quasi-emotional phenomena there was, correspondingly, little change in blood sugar, or a fall. Furthermore, in three other cases in which decortication caused great activity and a remarkable rise of blood sugar, a second brain section just in front of the

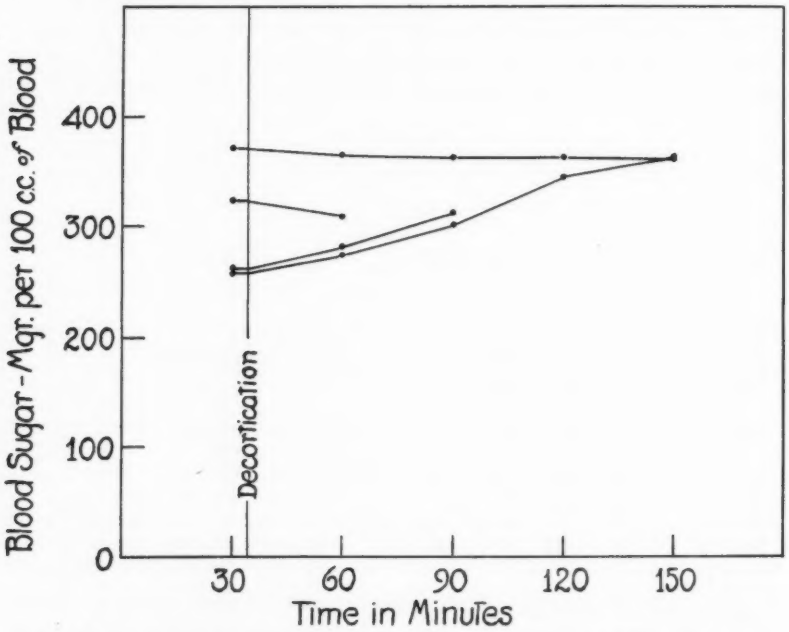

Fig. 2. Blood sugar in decorticate animals in which no pseudaffective phenomena occurred.

bony tentorium, that abolished the pseudaffective condition and made prominent the condition of rigidity, was followed by a drop in the glycemic percentage. The change thus induced was in sharp contrast to the continued rise during two hours, that was observed April 3 and May 15 (table 1), when aspects of the quasi-emotional reaction were continuously present. The failure of blood sugar to rise sharply in decorticate animals which do not display quasi-emotional activity may be interestingly related to the observation by Cannon and Britton that when rigidity was prominent medulliadrenal secretion, as indicated by the

rate of the denervated heart, was much less than when the outstanding feature was the pseudaffective state (3).

We have not thought it necessary to increase the number of cases in the group represented by table 2 and figure 2, because a number of other investigators have reported on the glycosuria and in some instances on the hyperglycemia of animals after simple decerebration. Olmsted and Logan found that a guillotine decerebration in front of the corpora quadrigemina induced a high blood sugar which was maintained and might even rise (5). Their results (as shown in their table 1) correspond closely in magnitude and development with our results as reported in table 2, but not with those in table 1. Bazett, Tychowski and Crowell describe an animal in which the glycemic percentage a half-hour after a guillotine decerebration was 0.37; from that level it gradually fell, during 7 hours, to 0.20; the fall was not due to lack of available glycogen, for in a terminal asphyxia the blood sugar rose to 0.38 per cent (6). Mellanby alone has reported a case in which the blood sugar rose after "decerebration" in a manner resembling that shown in figure 1. In his case the rise was from 0.46 per cent to 0.52 in 1 hour, to 0.62 in 2 hours, and to 0.7 in 3 hours; thereafter the percentage fell (7). His method of decerebration, however, was that of injecting starch grains into one carotid artery—a method which, as Bazett has remarked, leaves very uncertain the actual area of brain damage. It may be that in Mellanby's case some pseudaffective activity was present. In all the other cases mentioned above, however, the results correspond to those in our table 2, but not to those in our table 1—there was no sharp increase of blood sugar, with continued and rapid further rise, such as we have seen in the pseudaffective state.

TABLE 2

Blood sugar in decerebrate animals in which no pseudaffective phenomena occurred

DATE	BLOOD SUGAR (MGM. PER 100 CC. OF BLOOD)				
	Before decortication	After decortication (approximately half-hour stages)			
March 1	322	310			
March 4	261	281	312		
April 1	371	366	363	363	361
May 22	258	274	301	344	363

The medulliadrenal factor in pseudaffective hyperglycemia. The two groups of cases above described led us to expect that if pseudaffective phenomena were evident a high blood-sugar percentage would develop promptly after decortication, and that it would rise to higher levels with the passage of time. When these experiments were performed we had not

the definite proof which we now have (3) that under the circumstances medulliadrenal secretion is increased. We suspected that it was increased however, and accordingly we made tests to determine whether or not the presence and activity of the adrenal glands played an important rôle in the hyperglycemia of the pseudaffective state. We made these tests in two groups of experiments: one group in which the pseudaffective state was induced after the adrenal glands had been removed,

TABLE 3

Blood sugar in animals without adrenal glands or with the right adrenal gland removed and the left splanchnic nerves severed

DATE	BLOOD SUGAR (MGM. PER 100 CC. OF BLOOD)						REMARKS
	Before decortication		After decortication (approximately half-hour stages)				
1924							
April 9	279*	266	221	208	191	161	Some struggle, tail slightly bushy
April 10	434*	500	400	330	322	312	Claws showing, tail bushy, paws wet, very active
April 23	380*	502	478	388	351	333	Blood sample no. 2 dark. Same phenomena as on April 10. Blood pressure 130 to 80 mm. Hg
April 24	328*	339	280	279	233	190	Same phenomena as on April 10. Blood pressure 120–110
May 3	†	211	181	162	161	154	Tail wagging, paws moist. Blood pressure 110–140. Adrenalin (0.5 cc., 1:1000) increased blood sugar 154 to 235
May 7	†	152	148		148	131	Tail bushy, wagging, claws showing, very active. Blood pressure 156–140
May 10	†	281	232	232	224	200	Same phenonena as May 7. Blood pressure 158–122
May 17	†	310	308	289	270	248	Same phenomena as May 7. Adrenalin (0.5 cc., 1:1000) increased blood sugar 248 to 351 in half hour

* Adrenal glands removed.

† Right adrenal gland removed, left splanchnics cut February 14, 1924.

or after one had been removed and the nervous control of the other largely destroyed by section of the splanchnics; and the other group in which the state was induced after section of the nerves supplying the liver but without disturbance of adrenal function.

Table 3 and figure 3 present the results of observations on animals in which adrenal participation in the quasi-emotional response was either wholly abolished, or was greatly interfered with. In the former category are the first four cases of table 3. Blood samples taken just before

and just after removal of the adrenal glands, showed an increase of the sugar content in all but one animal. Thereupon decortication was performed. The pseudaffective phenomena did not occur quite so soon in these instances as they did when the operative procedures took less time and the etherization was therefore less deep and less protracted. Nevertheless, before the end of the first half-hour after the decortication,

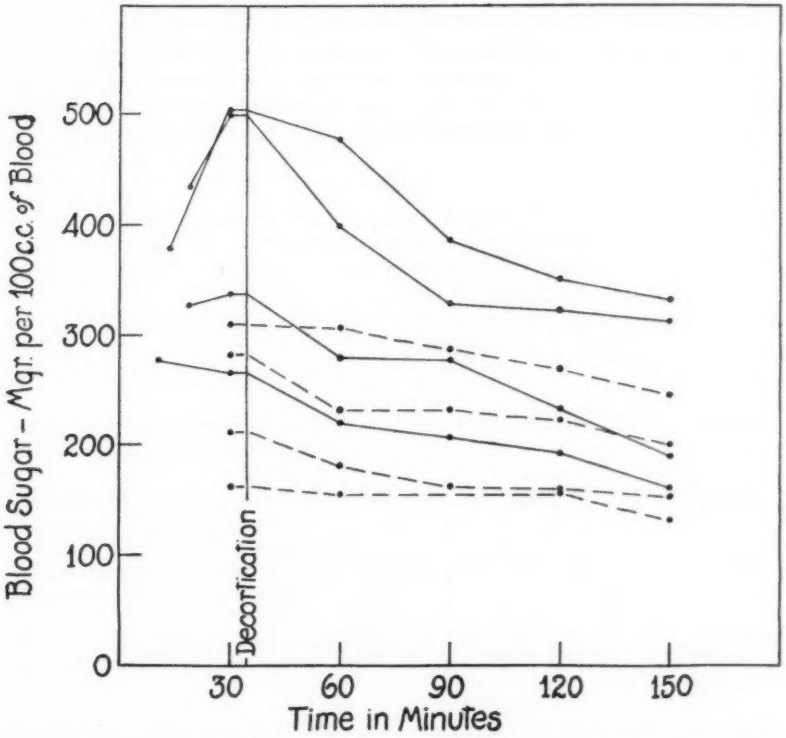

Fig. 3. Blood sugar in decorticate animals manifesting pseudaffective phenomena; without adrenal glands (continuous lines), or with the right adrenal gland removed and the left splanchnic nerves severed (dash lines).

the signs of sympathetic discharge were well marked. Although the animals had bushy tails and sweaty paws and were active, the blood sugar, instead of increasing as it did in the cases reported in table 1 and figure 1, invariably fell to a lower level. We would emphasize that the animals were in quite as good nutritive condition as those recorded in table 1, and that adrenalectomy required only a short period—12, 8, 13 and 10 minutes respectively. One might argue, however, that immediate

abdominal operation, which was performed in these first four cases of table 3, was in itself, apart from exclusion of the adrenal factor, the occasion for the subsequent drop in blood sugar. The last four cases of table 3, however, do not favor that interpretation. In the latter series the right adrenal gland had been removed and the left splanchnics cut eleven to thirteen weeks previously. Although there are, besides the splanchnics, other small strands from the sympathetic chain that influence adrenal secretion and that were not cut, they were not affective in these instances. Only the quick procedure of cannulating one carotid and tying the other was performed before the decortication. The quasi-emotional state was manifest very soon thereafter, and was of marked intensity. In no case, however, did the glycemic percentage rise; in-

TABLE 4

Blood sugar in animals, with liver denervated and with or without adrenal glands, that were decerebrated and manifesting pseudaffective phenomena

DATE	BLOOD SUGAR (MGM. PER 100 CC. OF BLOOD)						REMARKS
	Before decortication		After decortication (approximately half-hour stages)				
1924							
April 7	250*	241	218	177	151	122	*Adrenals removed, liver denervated. Very active, paws moist, tail bushy
April 8	380*	416	470	476	465	421	*Liver denervated, adrenals in. Claws showing, paws moist, active
May 13		307	348	400	412	444	Liver denervated 7 days before. Adrenals in. Rigidity, occasional activity
May 14		298	314	354	421	459	Liver denervated 8 days before. Adrenals in. Considerable activity

stead, in every case it ultimately fell. There was nothing to indicate that the fall was due to injury of the pituitary; 6 of the 8 cases were decorticated by way of the orbits and post-mortem examination revealed no disturbance at the base of the brain. There was nothing to indicate that the fall was due to lack of mobilizable sugar: the animals had been living comfortably in the laboratory for many weeks and were well fed and plump; and furthermore, in two instances (May 3 and May 17) adrenalin was injected subcutaneously after two hours, whereupon the blood sugar promptly and markedly increased. Obviously the reserves were adequate and ready to be called forth. The results in the last four cases should be compared with those in the first four cases of table 3. Whether

the splanchnic nerves were intact on one side or on both sides made little difference so long as the adrenal glands were absent or inactive.

The importance of the adrenal factor in the mobilization of sugar in the blood may be revealed also by destroying the nerve supply to the liver and leaving the adrenal glands innervated. Since the pseudaffective state is characterized by increased medulliadrenal secretion, and since

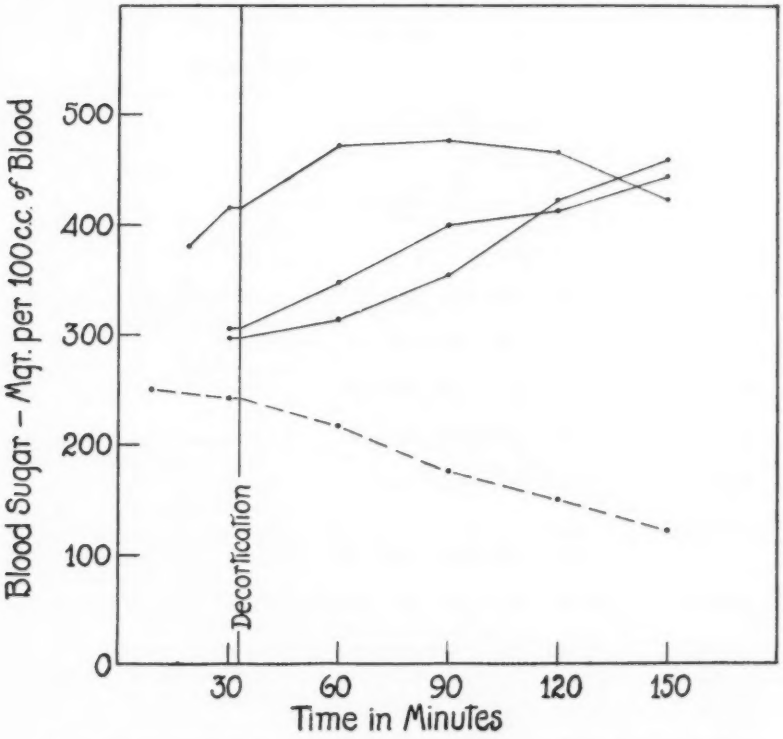

Fig. 4. Blood sugar in decorticate animals in the pseudaffective state; with liver denervated and adrenal glands present (continuous lines), and adrenal glands absent (dash line).

nerve impulses are no longer effective on the liver, the hepatic cells are now influenced only by the humoral agent. In table 4 and in figure 4 are shown the results of observations on animals with the liver nerves severed. They were cut along with the duodenal nerves on the duodeno-hepatic artery. In conformity with the cases shown in table 3, the first case of table 4, in which not only were the hepatic nerves cut, but also the adrenal glands were removed, a high degree of quasi-emotional dis-

play was accompanied by a fall instead of a rise in the glycemic percentage. In the other cases, however, hepatic denervation, whether done in the acute experiment or earlier, did not check the routine rise of the blood-sugar level that is characteristic of the onset of the pseudaffective state.

By comparing figures 1, 3 and 4 the evidence is clearly revealed that when an animal is exhibiting sham rage the adrenal glands play an important rôle in determining the amount of sugar in the blood. If they

TABLE 5

The effect of insulin in decerebrate animals

DATE	UNITS OF INSULIN PER KILO	BLOOD SUGAR (MGM. PER 100 CC. OF BLOOD)						REMARKS
		Before decortication	After decortication (approximately half-hour intervals)					
1924								
February 28	4	396	425*	389	255	174	158	Tail bushy, paws wet, claws showing, very active
March 1	4	322	310*	233	189	161	134	Animal quiet. Clots over and in brain
March 4	2	261	281	312*	304	267		Animal quiet. Clots over and in brain
March 5	2	388	408	430*	392	323		Animal active, paws sweating. B.p. 190–140 mm. Hg.
March 10	2	266	333*	266[1]	190			B.p. 136–80. [1]Adrenals removed (after 266)
March 22	1	266[1] 270	339*	235[2]	88	68		[1]Right adrenal removed and [2]left splanchnics cut. B.p. 160–120. Some activity
March 26	4.6	357	384*	269[1]	217	157	118	[1]Adrenals removed. Activity. B.p. 160–94
May 27	0.5	292	434	434	476*	339	255	Paws wet, clawing, very active. Sugar fell to 212 next sample

* Insulin was injected immediately after this blood sample was taken.

are present they are active, and then the sugar content rises whether or not the hepatic nerves are intact; if the glands are absent or inactivated the sugar content falls.

The effectiveness of insulin in decorticate animals. In table 5 and figure 5 are reported the results of giving various doses of insulin (Lilly) to decorticate animals which in the main were displaying quasi-emotional phenomena. The early stages of the observations on March 5 and May 27 appear also in table 1, and those on March 1 and March 4 appear also in table 2. With the exception of one of the latter cases decortica-

tion caused in all the animals the usual increase of the sugar content of the blood and in three instances the content continued going higher for an hour or an hour and a half until conditions were changed by the administration of insulin. The insulin, which was injected intravenously, varied in dosage from 0.5 to 4 units per kilo. Without regard to the amount injected the blood sugar was invariably brought lower by it.

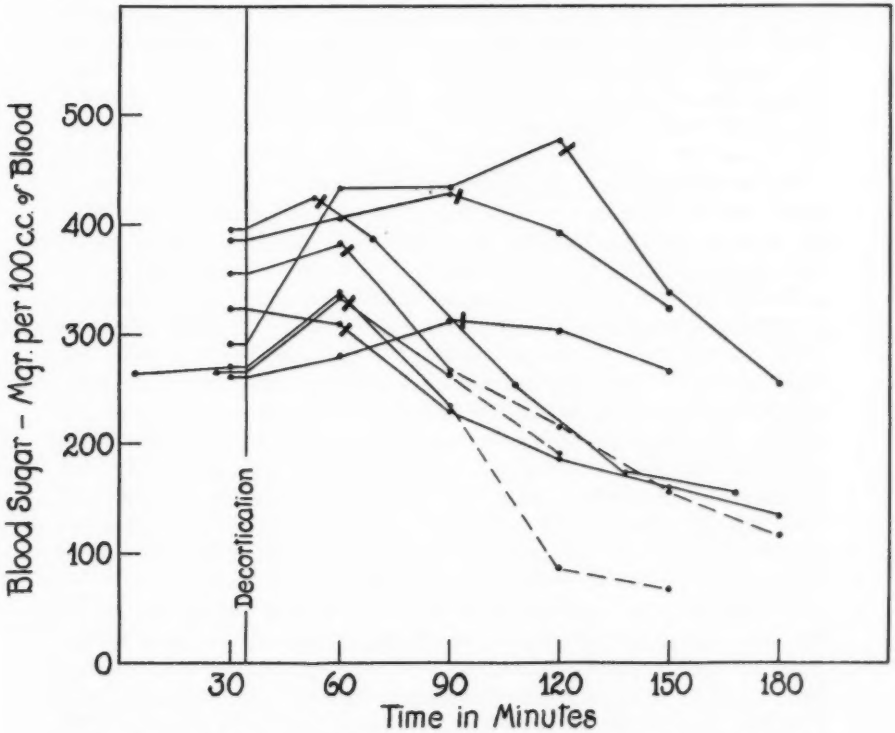

Fig. 5. The effect in pseudaffective hyperglycemia of giving insulin (at times indicated by broad cross lines). Dash lines indicate that adrenals were removed or in activated.

At a late stage in three instances (March 10, 22 and 26) the adrenal glands were excised or rendered less active by splanchnic section; the dash lines of figure 5 mark the period of the experiments when this factor was excluded. The evidence is too slight to warrant a general conclusion regarding removal of splanchnic and adrenal influence on the action of insulin; it may be noted, however, that in two of the cases the rate at which the sugar content of the blood was falling was not hastened to

a noteworthy degree; and in the third case reduction of the remaining medulliadrenal activity by cutting the splanchnic nerves was followed by a much faster rate of falling than had prevailed before. In general the result to be emphasized in these experiments is that the high blood-sugar level was reduced by insulin, in spite of the quasi-emotional condition of the animal.

The statement has been made that if cats are decerebrated and the pituitary body is left intact a high blood-sugar level is maintained, which is not materially reduced by insulin (5). We were unable to discover in our animals evidence that the pituitary body had been damaged by our decortication. In all but one of the cases reported in table 5 (that of March 10) orbital decortication was performed. Though clots were found over the hemispheres and in the ventricles in two cases (March 1 and 4), extensive bleeding was rare, the pituitary body was left uninjured, and as indicated by the prominence of spontaneous activity and the signs of the pseudaffective state, the blood supply to the region of the mid-brain and in front of it was quite adequate. According to our evidence the drop of the blood-sugar percentage after insulin administration was not made possible by impairment of the pituitary gland.

Discussion. *The importance of the adrenal medulla in hyperglycemia.* The increase of blood sugar under the conditions described in the foregoing pages was in all probability largely if not wholly the result of stimulation of the glycogenolytic process in the liver. At least there is no reason to suppose that sugar utilization was impaired, and that in consequence an accumulation occurred; and there is abundant evidence that when the splanchnic nerves are stimulated, as they are in the pseudaffective state (shown by augmented medulliadrenal secretion (3)), sugar is liberated into the blood from the hepatic stores.

The glycogenolytic process in the liver is normally subject to two influences: it may be stimulated both by nerve impulses and also by adrenin in the circulating blood. The relative importance of these two factors has been the subject of extensive enquiry, but the results have varied so greatly that considerable confusion exists, in the midst of which controversy is rife. The main question at issue is whether medulliadrenal secretion plays an essential or even an important part in mobilizing sugar from the liver.

Prominent among those who have denied that the adrenal glands are significant in the production of hyperglycemia are Stewart and Rogoff. "The best evidence," Stewart writes (8), "that the epinephrin output exerts no important or indispensable function is that after its suppression the animals do not differ notably from normal animals in their blood sugar content, in their power to form glycogen, their power to mobilize sugar in response to piqûre, etc." Of this argument that medulliadrenal

secretion is useless, the part with which we are now concerned is the last. The reference to "piqûre, etc." presumably includes the other observations by Stewart and Rogoff, on the efficacy of ether anesthesia and asphyxia as means of increasing the glycemic percentage although the adrenal glands have been removed. On these observations Stewart bases his conclusion.

In testing whether medulliadrenal secretion plays a rôle in the hyperglycemia accompanying etherization and asphyxia Stewart and Rogoff eliminated the adrenal factor in their first series of experiments by removing one gland and cutting the nerves of the other, and in their second series by excising both glands (9). Under these conditions etherization and asphyxia produced a moderate increase of blood sugar, and they concluded that the adrenals were unnecessary for that result. Others have shown, however, that the hepatic nerves also are not necessary. King and his co-workers severed all the splanchnic fibers of the celiac plexus going to the liver and found that the hyperglycemia elicited by ether was not prevented (10). And Keeton and Ross observed that although the nerves on the hepatic artery had been cut ether induced a hyperglycemia quite equal to that in animals with the hepatic innervation intact. Even when the splanchnic nerves had been cut, so that both the nervous and the humoral factors acting on the liver were almost completely set aside, ether evoked a well-marked rise in the glycemic percentage (11). Fujii has reported similar observations (12). What is true of the action of ether is true also of asphyxia. Macleod cut the fibers of the hepatic plexus and yet was able to produce by mechanical asphyxia a noteworthy hyperglycemia (13). And Kellaway has reported that after splanchnic section and subsequent removal of both adrenals asphyxiation can still cause a large increase of blood sugar (14).

The hyperglycemia provoked by ether or asphyxia after the splanchnic nerves have been cut (and in some instances after the adrenals also have been excised) proves too much for Stewart's limited inference. It shows, to be sure, that the adrenals are unnecessary. It also shows that the nervous influences are unnecessary—indeed, that ether and asphyxia can cause liberation of sugar into the blood by affecting the liver cells directly. Ether and asphyxia are agents capable of general action throughout the body, and do not necessarily operate by way of the nervous system. From experiments made with them the general statement that the adrenal factor is not important for glycogenolysis is quite as unwarranted as would be the general statement that the nervous factor is not important for that process. Since both factors may be excluded the experiments have no bearing on the relative importance of either. In these circumstances to draw a conclusion regarding only one of them, as Stewart and Rogoff have done, is only partial and illogical.

In some of the experiments cited, however, not all the nerves to the liver were excluded; the splanchnics had been cut, to be sure, but in addition there are small strands passing from the lumbar sympathetic chain to the semilunar ganglion. One may argue, therefore, that though the nerve supply to the liver was seriously crippled, it was not *wholly* destroyed. And since the nervous factor may still be at work, the foregoing strictures on Stewart's conclusion, one may say, are not justified. But in certain of the experiments mentioned above, the hepatic nerves themselves were carefully sectioned, and yet the blood sugar was increased by ether and asphyxia. Now either these agents have acted directly on the hepatic cells, in which case the foregoing comments are pertinent, or they have acted in some indirect manner. Inasmuch as the nervous control of the liver has been eliminated, however, the remaining mode of inducing glycogenolysis is by means of medulliadrenal secretion. But if ether and asphyxia act by increasing that secretion Stewart's argument for uselessness is at once disproved. Neither group of experiments, consequently, supports Stewart's contention.

The remaining series of experiments used by Stewart to prove the insignificance of secreted adrenin in mobilizing hepatic glycogen are concerned with the effects of piqûre. In 1913, Freund and Marchand (15), working on rabbits deprived of the adrenal glands, saw in a number of cases a considerable increase of blood sugar after piqûre, but inasmuch as the experiments were performed under ether, which itself causes hyperglycemia, their results are questionable. In 1918, Stewart and Rogoff (16) reported on five adrenalectomized rabbits in which they made a sugar puncture: in one the maximal glycemic percentage was 0.176 (they refer to 0.162 per cent as doubtful); in two it rose above this level (to 0.205 and 0.449 per cent); and in the other two the results were quite negative. Their argument therefore is based on two cases out of five in which after adrenalectomy piqûre was followed by a significant rise of blood sugar. Even in these cases there is no record of respiration or blood pressure to prove that the increased glycemia was not due to asphyxia. But on the assumption that an asphyxial factor was absent, these two cases show that it is possible for piqûre to increase the sugar content of the blood when the adrenal glands are absent. They do not justify, however, Stewart's conclusion that secreted adrenin is of no importance to the organism. For when the glands are present an effective medulliadrenal secretion might result from the piqûre and by coöperating with the nervous factor might hasten or increase the sugar output from the liver. Stewart and Rogoff have removed the humoral factor and, finding that the nervous factor still does *something*, they conclude that the humoral factor has no important function. They have not reversed the

procedure, i.e., they have not removed the nervous factor and tested what the humoral factor could do.

That there is a large increase in medulliadrenal secretion in consequence of piqûre was definitely shown in this laboratory in 1922 by Carrasco-Formiguera (17); and since then Trendelenburg has described experiments showing that this increased secretion is quite adequate by itself to produce hyperglycemia (18). Corresponding testimony was given by Griffith, who found that reflex hyperglycemia, after denervation of the liver, was practically as great as in normal animals, but if the adrenal glands were removed or inactivated, reflex hyperglycemia, though still considerable, averaged less than in normal animals (19). Quite in accord with this evidence is that offered in the present paper—the pseudaffective activities are associated with increase of blood sugar if the adrenals are present (cf. fig. 1), even though the hepatic nerves have been cut (cf. fig. 4), but if the adrenal glands have been removed or inactivated, even though one or both splanchnic sets still supply the liver, pseudaffective activity is attended by a failure to hold the high glycemic level prevailing at the start of the experiment (cf. fig. 3). These various comments and observations, wholly consistent among themselves, are quite opposed to Stewart's reasoning that medulliadrenal secretion is of no importance to the organism. Indeed, the evidence obtained by use of the pseudaffective preparation, as shown by contrasting figures 3 and 4, indicates that of the two factors influencing glycogenolysis the humoral is more important than the nervous.

Throughout their experimental work on hyperglycemia, and in the discussion of the results of it, Stewart and Rogoff have failed to consider what is known in logic as "plurality of causes." There is good evidence that both nerve impulses and secreted adrenin can act on liver cells and liberate sugar into the blood. In their attempt to eliminate the adrenal agency Stewart and Rogoff have used Mill's "method of agreement," i.e., that an agent is not the cause of a phenomenon if the phenomenon occurs in its absence. It is well recognized, however, that this method can not properly be applied when there are plural causes (20). Yet into just this fallacy have Stewart and Rogoff fallen. By such reasoning as they have employed one can argue that it is the *left* vagus, the *left* splanchnic, the *left* kidney, the *left* testis, "which exert no important function," to recall Stewart's phrase, because, after suppression of these *left* members "animals do not differ from normal animals." Before drawing that conclusion might it not be prudent to remove the *right* member in each instance, instead of the left, to learn whether in fact it is the *left* member which is of no importance? A quite similar situation, presented by the nervous and humoral factors which affect numerous

bodily functions, affords dangerous opportunities to slip into the error of neglecting alternative causes. That error appears not only in conclusions drawn by Stewart and Rogoff, but also characterizes much of the reasoning of Gley and Quinquaud regarding the function of the adrenal medulla.

The initial hyperglycemia. The foregoing discussion has emphasized the importance of etherization and asphyxia as conditions which produce hyperglycemia. Added to these another highly potent condition for glycogenolysis is emotional excitement—a condition carried to the extreme in its physiological aspects in the pseudaffective state. When an animal is very quickly etherized, by means of a cone closed by ether-soaked cotton, all three of these conditions are present and coöperating. There is little to wonder at, therefore, that the height of blood sugar at the start of our experiments was often much above that found in normal quiet existence. Even in the case reported in table 4 and figure 4, in which the hepatic nerves were cut and the adrenals were inactivated, the initial glycemic percentage, though not nearly so high as in cases of normal innervation, was elevated above the basal level. In that case emotional reactions, which would be influential by nervous channels, probably played a minor part, if any part at all; but ether and asphyxia, which, as we have seen, can act directly on the liver cells, would satisfactorily account for the increased sugar content of the blood.

The question of pituitary involvement in decerebrate hyperglycemia. In 1923 Olmsted and Logan reported that decerebrate cats with the pituitary body intact maintain a high blood-sugar level which is not materially reduced by insulin; that if the pituitary body has been removed, the initial hyperglycemia is not maintained and typical insulin convulsions can be induced; and that the presence or absence of the adrenal bodies does not appear to have a marked influence on these results (5).

Olmsted and Logan recorded blood sugar in six cases of decerebration in which the pituitary was left intact. In four of them the percentage increased to a moderate degree (the maximum was 0.4, which was reached 4 hours after decerebration); in the other two it was maintained for several hours at approximately the original elevation. If these results were plotted, the lines would correspond closely to ours as shown in figure 2. We have already noted that in such cases, lacking quasi-emotional signs but usually manifesting rigidity, medulliadrenal secretion and sugar mobilization are quite moderate as compared with what occurs when the sympathetic system is being strongly excited in a sham rage (see p. 298). If the pituitary is removed after decerebration, the blood sugar, according to Olmsted and Logan, does not rise or even maintain its high level. Only one uncomplicated case in their series, however, supports this statement. Even if there were numerous supporting instances, how-

ever, it is questionable whether the drop in the glycemic percentage should be ascribed to the pituitary body, or to pituitrin, as Macleod has suggested (21). There is good evidence that in the floor of the third ventricle just above the pituitary (in the "hypothalamus") is a region having important control over functions involving activities of the sympathetic system (22). Keeton and Becht were able to cause hyperglycemia by stimulating the pituitary, even after section of the nerves of the hepatic pedicle, but not if the splanchnic nerves were cut. They attributed the effect, therefore, not to circulating pituitrin, but to nerve impulses acting on the adrenals and the liver cells. Extirpation of the gland caused a transitory hyperglycemia, lasting 3 to 5 hours, and followed by a persisting normal level (23). All these results can be simply explained if we assume *a*, that, when the "pituitary" is stimulated electrically, the current reaches the nerve centers directly above it and causes them to discharge impulses into sympathetic channels, and *b*, that, when the pituitary is removed, unless very great care is exercised, these centers are likely to be damaged and thereby rendered functionally defective. Until that interpretation of their result is ruled out Olmsted and Logan are not justified, in our opinion, in attributing the ups and downs of blood sugar after decerebration to the presence or absence of the pituitary body.

As already noted, the pituitary body was intact in our cases of decortication. Nevertheless, insulin caused a marked drop in the glycemic percentage (cf. table 5 and fig. 5). It likewise caused a drop in the three cases with intact pituitary in which Olmsted and Logan used it—from 300 to 160 mgm. in 2 hours, from 370 to 285 mgm. in 1 hour, and from 240 to 200 mgm. in 3 hours. In the two cases ascribed to absence of the pituitary body, which they report in full, the drop was from 212 to 80 mgm. and from 170 to 84 mgm., in 2 hours. Of all except the third of these five cases the results, when plotted, correspond closely to those in our figure 5, and reveal nothing peculiar in the effect of insulin whether the pituitary is present or not. In both conditions the *rate* of fall in the glycemic percentage was within the limits of ordinary variations. To be sure, a convulsive level was reached after a few hours in animals without the pituitary, but in them the initial blood sugar was already low. To suggest on the basis of these few cases, which are not decisive, that the high blood sugar after decerebration is maintained by pituitary influence, seems to us not warranted.

Olmsted and Logan's conclusion that the adrenal bodies do not have a marked influence on the results they obtained is founded on two cases in which they removed the pituitary body and tied off the adrenals. In both cases the glycemic percentage slowly fell. But in the only control case they report, in which the pituitary alone was removed, the percentage

likewise slowly fell (for four hours). They have made two changes, removed the pituitary and the adrenals (each resulting in a drop in the blood sugar), and have drawn a conclusion about one of them—that adrenalectomy does not have a marked influence. They could not have expected adrenalectomy to *increase* the glycemic percentage—all we know of the effects of adrenin is opposed to that supposition. If they expected it to *decrease* the percentage, why was the test made on a preparation in which, according to their evidence, the percentage would decrease anyhow? It seems to us that the proper mode of showing whether the adrenal glands are important in maintaining the hyperglycemia after decerebration is by excluding them when conditions favor continued hyperglycemia, in order to see whether in fact it is continued. This we have done, and we find, contrary to Olmsted and Logan, that in decerebrate animals with intact pituitary the adrenal glands are essential to the maintenance of the high blood-sugar level (cf. table 3 and fig. 3).

SUMMARY

The pseudaffective state described by Cannon and Britton is attended by a hyperglycemia which increases as time passes (see fig. 1). If decerebration is not followed by pseudaffective activity the glycemic percentage does not increase markedly and may fall (cf. figs. 1 and 2).

If the adrenal glands have been removed, or if one has been removed and the splanchnic supply of the other has been destroyed, the initial hyperglycemia of the pseudaffective state neither rises nor remains high; instead it declines (cf. figs. 1 and 3).

If the hepatic nerves have been severed without disturbance of the adrenal glands the initial hyperglycemia is increased during quasi-emotional activity.

Insulin is effective in reducing the hyperglycemia that follows decortication, even though pseudaffective phenomena are prominent and the pituitary body is intact.

The relation of the adrenal medulla to hyperglycemia is discussed, and the weak evidence and the erroneous logic leading to the conclusion that it plays no important rôle, are pointed out. Also the claim that there is pituitary involvement in decerebrate hyperglycemia is questioned and reasons are advanced for discrediting that claim.

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THE COMPARATIVE EFFECTS OF ADRENALIN INFUSIONS ON BLOOD PRESSURE AND GASTRO-INTESTINAL MOTILITY

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Langley in 1901, while studying the action of various poisons upon sympathetic cells, observed that injections of adrenalin produced upon cats, dogs and rabbits effects that "are almost all such as are produced by stimulation of some one or other sympathetic nerve" (1). Following the report of this work there grew up the "tonus theory" of adrenal function which assumed that the presence and action of adrenalin "is essential for the maintenance of normal arterial tone, and, indeed, for the tonic activity of all muscles innervated through the thoracic sympathetic nerves" (2). In 1905 Elliott confirmed the findings of Langley and localized the action of adrenalin in the myoneural junction (3). The results reported by both Langley and Elliott were secured with injections of adrenalin which, though within the non-toxic limits, were not otherwise restricted as to quantity.

To prove the applicability of these results to the normal function of the adrenals it would be necessary to show that the effects reported could be produced by amounts of adrenalin such as are normally elaborated by the glands. Hoskins and McClure have reported evidence "that the adrenals do not produce more than one-fourth enough secretion to affect blood pressure" and that when adrenalin is injected into the circulation in amounts comparable to the concentration normally existing in blood the result is a fall instead of a rise of arterial pressure. They concluded that adrenal secretion could not be an immediate factor in vasomotor tonus (4).

To investigate further the applicability of Elliott's results to normal conditions, Hoskins and McClure compared the simultaneous effects of adrenalin upon blood pressure and peristalsis. It was reasoned that if adrenalin is a factor in maintaining vascular tonus pressor effects would be elicited by concentrations less than would evoke depression of intestinal peristalsis; otherwise the mechanism would be detrimental to an essential body function. Their work was done upon dogs and the injections were short lasting, from 1 to 2 minutes. It was found that "intestinal depression is caused by injections of epinephrin decidedly smaller in

amount than those requisite to cause increase in blood pressure" (5). They concluded, therefore, that normal vasomotor tonus is not dependent upon a minimal stimulating effect of circulating epinephrin.

It is conceivable, however, that the intestinal inhibition observed by these investigators is only a short-lasting phenomenon. The possibility was not excluded that the intestine might soon lose its irritability to adrenalin and that after a period of adjustment pressor effects could be obtained without inhibition of intestinal activity. It was decided to put this possibility to experimental test and to extend the study to include other animals than dogs. For this purpose the cat and rabbit were selected.

In the earlier experiments urethane anesthesia was used on rabbits and ether on cats and dogs. Later, with all animals better results were secured by first anesthetizing with ether and maintaining the anesthesia with urethane—2 grams per kilo body weight administered by stomach.

The most consistent results were obtained when 15 to 30 minutes were allowed to elapse between the completion of the operation and the beginning of the observations. In this way the depressing reflex effects of sensory stimulation were sufficiently obviated. The practice of pithing the cord to eliminate splanchnic inhibition of the intestine was attempted and abandoned early in the research because of the resultant low blood pressure, it having been shown that amounts of adrenalin which depress normal or high blood pressure give the reverse effect if the pressure is lowered by other means (6).

In all the experiments blood pressure was recorded by means of a mercury manometer connected with the right common carotid artery. Various methods for recording gastro-intestinal activity were used. The most satisfactory from the view point of ease of manipulation, delicacy of response and the minimizing of sensory stimulation was the simple apparatus described by Alvarez (7).

Adrenalin chloride (Parke, Davis & Co.) was used in dilutions varying from 1:250,000 to 1:2,000,000, depending on the size of the animal. Since the experiments were long lasting and a small total amount was used the adrenalin was diluted with distilled water, thus obviating the destructive effects of the salts contained in the menstrua commonly used in short lasting experiments. The adrenalin was injected into the left external jugular vein. A Luer needle which had been cut off and upon which a small knob of solder was placed proved to be the most convenient form of injecting cannula. Two forms of perfusion apparatus were used. A Mariotte bottle suspended from a windlass worked very well, but when used in experiments upon large animals the elevation had to be adjusted frequently to accommodate changes in venous pressure. The Woodyatt pump was used in later experiments with greater facility but with no more accuracy since the rate of output changed with variations in the strength

of the electric current, and this in turn fluctuated with variations in the street line load. This factor necessitated the use of the "trial and error" method for determining the rate at which the perfusion should be made. It occasionally happened that this required so much time that in order to eliminate a new variable the experiment had to be discontinued, Battelli and others having shown that with repetition the threshold of stimulation rises (8). From 0.1 cc. to 2.0 cc. per kilo per minute of perfusate was used with an average amount of 0.5 cc. per kilo per minute. This amount of fluid was shown not to affect the blood pressure under the conditions of these experiments.

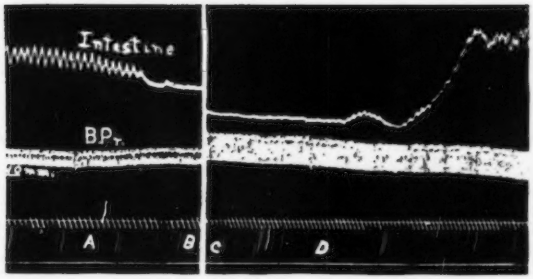
In a few experiments gastric peristalsis was studied, but as a rule the intestine was used. With cats and dogs the serrefines were attached through the omentum which thus afforded a natural protection from drying. The exposed area was well protected with cotton, the recording and perfusing apparatus adjusted and the animal left undisturbed until the gastro-intestinal activity had reached a consistently active state. The minimal amount of adrenalin required to produce a pressor effect was then determined and observations were repeated as long as blood pressure and respiration remained normal.

In all, 39 observations were made upon 10 dogs; 16 observations upon 4 cats, and 53 observations upon 13 rabbits. The effects of adrenalin upon the organs studied were found to be consistent within the species, and to be the same upon cats as upon dogs, but the effects upon cats and dogs differed markedly from those upon rabbits.

The observations made upon dogs confirm the findings of Hoskins and McClure for short lasting injections and show that no new factor is introduced when the perfusion is continued for forty minutes, that is, the constant perfusion of adrenalin in amounts too small to affect blood pressure produces complete stasis of the intestine which lasts as long as the perfusion is continued (fig. 1).

Cats with both short- and long-lasting perfusions of adrenalin chloride gave essentially the same response as did dogs.

In case of rabbits very small amounts of adrenalin chloride (0.00026 mgm. per kilo body weight per minute) caused a minimal rise in blood pressure with no effect upon the intestine (fig. 2). It is interesting to note that the amount used to produce this effect is only slightly greater than is estimated by several investigators to be normally discharged by the adrenals (9). Amounts sufficient to raise and maintain the blood pressure 8 or 10 mm. above normal for a considerable period of time (30 to 45 minutes) had a slight depressant action upon the activity and tonus of the intestine from which it always recovered in 5 to 15 minutes after the injection was begun (fig. 3). Amounts sufficient to raise and maintain the blood pressure 30 mm. above normal had an immediate strongly de-



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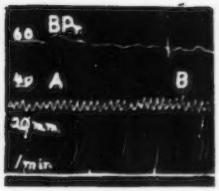

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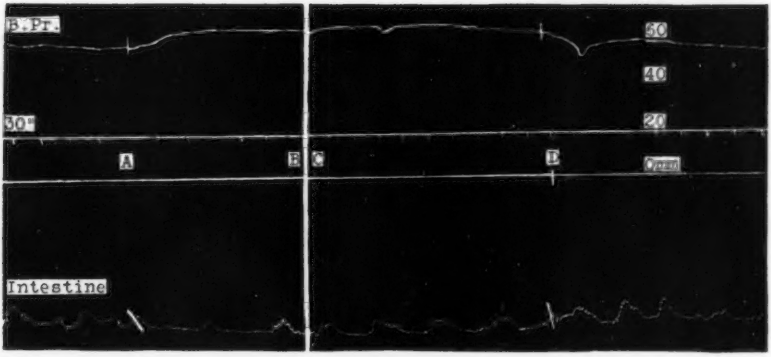

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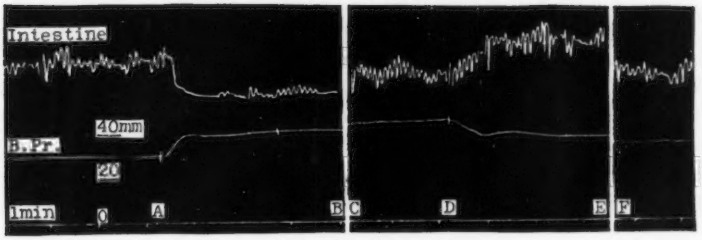

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Fig. 1. Dog, 4 kilo. Lumbar and thoracic cord pithed. Ether anesthesia Between *A* and *D* adrenalin chloride 1:500,000 was perfused for 40 minutes at the rate of 4 cc. per minute (0.002 mgm. per kilo per minute). From *B* to *C* 37 minutes of record was omitted which was essentially the same as from *C* to *D*. The change in amplitude of the blood pressure trace was due to removal of a clot from the cannula.

Fig. 2. Adult rabbit. Middle trace, rhythmic contractions of intestine with respiratory waves superimposed. From *A* to *B* adrenalin chloride was perfused at the rate of 0.00026 mgm. per kilo per minute.

Fig. 3. Adult rabbit. The intestinal trace shows rhythmic segmentations superimposed upon peristaltic waves. From *A* to *D* adrenalin chloride was perfused by vein at the constant rate of 0.0006 mgm. per kilo body weight per minute. Between *B* and *C* 8½ minutes of the record similar to that from *C* to *D* has been omitted.

Fig. 4. Rabbit, 2.7 kilo. Urethane anesthesia. The intestinal trace shows rhythmic segmentations superimposed upon tonus waves. From *A* to *D* adrenalin chloride was perfused at the rate of 0.003 mgm. per kilo per minute for 30 minutes. A portion of the record between *B* and *C* representing 25 minutes of the injection period is omitted. During the first fifteen minutes of this period there was a gradual change in blood pressure and in intestinal activity from the conditions represented at *B* to those represented at *C*. Between *E* and *F* 10 minutes have been omitted.

pressant effect upon the intestine from which, however, it showed the usual tendency to recover.

Three of the 13 rabbits gave depressor responses of blood pressure to injections of adrenalin chloride (fig. 5). In 2 of the 3 rabbits that gave this response it was produced by an amount of adrenalin chloride larger than that required to produce a minimal pressor effect in the same rabbit. These observations are contrary to those reported by other investigators (10). The response has not been observed often enough to allow conclusions as to its cause.

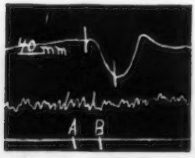

Fig. 5. Rabbit, 2.5 kilo. Urethane anesthesia. Upper trace, blood pressure. Middle trace, rhythmic segmentations of intestine. Lower trace, signal and O pressure. Between *A* and *B* 2.2 cc. adrenalin chloride, 1:2,000,000, were injected in 15 seconds

In half of the rabbits used a sensitization was produced by long-continued perfusions of adrenalin chloride so that activity of the intestine which surpassed the normal followed cessation of the perfusion. Whenever this sensitization occurred the intestine was observed to return to normal activity in from 2 to 10 minutes (fig. 4).

Our results consistently bore out the conclusion that the intestine of rabbits differs from that of cats and dogs in its response to adrenalin chloride. It is less sensitive to the drug than is blood pressure and its irritability quickly decreases so that it is able to escape from the initial inhibition. The data here reported show that as regards the cat and dog adrenalin cannot be a factor in maintaining normal blood pressure because vascular tone could be secured by such a mechanism only with the accompanying loss of a necessary nutritional function, namely, intestinal motility. On the contrary, it is demonstrated that such a mechanism could function in the rabbit.

SUMMARY

1. The simultaneous effects of long-lasting perfusions of adrenalin chloride upon the blood pressure and gastro-intestinal motility were recorded in 10 dogs, 4 cats and 13 rabbits.

2. It was found that with cats and dogs blood pressure could be raised and maintained at a pressor level only at the expense of gastro-intestinal motility. With these animals, therefore, the conclusions that normal vasomotor tonus is not dependent upon a constant stimulating effect of circulating adrenalin are confirmed and strengthened.

3. The generalization does not hold for rabbits since it is shown that in these animals the blood pressure can be raised and maintained slightly above normal by injections of adrenalin that are without significant effects upon the intestine.

4. Three of thirteen rabbits gave depressor blood pressure responses to adrenalin in amounts slightly larger than those required to produce minimal pressor responses.

5. Long-continued perfusions of adrenalin chloride cause a sensitization in the rabbit resulting in hypermotility of the gastro-intestinal tract when the perfusion is discontinued.

I wish to thank Prof. R. G. Hoskins for suggesting this problem and for helpful advice given during its solution. I am also indebted to J. R. Brandon and E. S. Hunter for technical assistance.

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THE EFFECT OF OBSTRUCTION OF THE HEPATIC VEINS ON THE SYSTEMIC CIRCULATION

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The hepatic circulation, receiving blood from the huge splanchnic region, is a most important element in maintaining the systemic blood pressure. Burton-Opitz (1) estimated that the equivalent of the total quantity of blood in the body passed through the liver every three minutes. Changes in the vascular bed which drains into the liver have been charged with being a factor in the production of shock. It has seemed, therefore, that a method of blocking the outflow of blood from the liver with a minimum of traumatism might throw some light on the relations between the hepatic and systemic circulations. We have devised a method by which it is possible to occlude the hepatic veins and thus to stop the outflow of blood from the liver, with relatively slight trauma, and a minimum of injury to important nerve plexuses and without material interference with the remainder of the circulation.

TECHNIQUE. The peritoneal cavity is opened by a median incision about three to four inches long, beginning at the xiphoid process. One end of a rubber tube about two feet long and about 0.5 cm. in diameter (in these experiments, a piece of Rehfus stomach tube was used) is passed along the right side of the falciform ligament between the liver and the diaphragm, through the membranous portion of the right triangular ligament, thence through the foramen of Winslow, then through the membranous portion of the left triangular ligament, and up along the left side of the falciform ligament between the liver and diaphragm. The only intraperitoneal injury is the perforation of the triangular ligaments, and, since this is done through the membranous portion, little hemorrhage results. A rubber tube is used in order to avoid tearing the friable liver. Care must be taken that all of the multiple lobes of the dog's liver are inclosed within the loop of rubber tube.

By exerting traction upward and somewhat caudad on the two ends of the rubber tube and at the same time pressing downward between the ascending parts of the tube with the finger, it is possible to constrict the hepatic veins just before they enter the inferior vena cava. By the simultaneous application of traction and pressure, the liver is not lifted

upward and there is little or no disturbance of the diaphragm and little or no constriction of the vena cava, and therefore no interference with the flow of blood to the right side of the heart from sources below the place of entry of the hepatic veins. That this is the case is made evident by the absence of any distention of the femoral veins, and also by the fact that substances, such as peptone, injected into a femoral vein while the hepatic veins are thus constricted, produce their effects promptly. Pulling upward on the rubber tube without downward pressure with the finger does interfere with the return flow of blood through the vena cava.

Discussion. Strong traction and firm pressure applied in the manner described above, cause a precipitate fall of from 35 to 60 mm. of mercury in arterial blood pressure. (See fig. 1.) The gross fall depends in part upon the degree of constriction of the hepatic veins, and in part upon

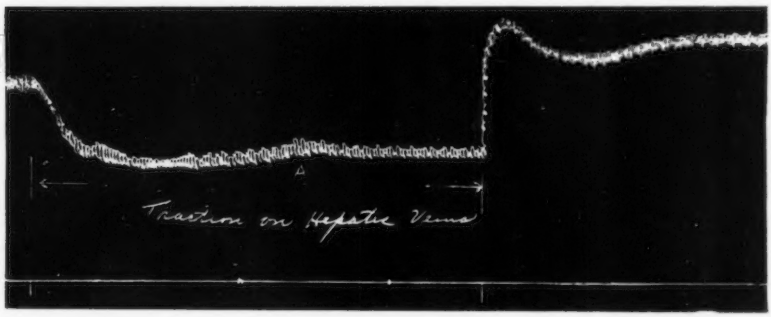

Fig. 1. The effect of obstruction of the hepatic veins on arterial blood pressure. Time 1½ minutes. The slight rise shown at *A* was due to a slight relaxation of traction and pressure on the veins.

the original pressure. The percentage fall in pressure appears to depend chiefly on the relative completeness of the occlusion of the veins. This ranged from 26 to 42 per cent of the arterial pressure preceding the experiment. In those instances in which it appeared that the obstruction of the hepatic veins was complete, the fall was between 37.5 and 42 per cent of the original pressure.

While the traction and pressure are kept constant the arterial pressure remains at a constant level for at least five minutes, the longest period during which constriction of the hepatic veins was maintained in these experiments. When the constriction is suddenly released the arterial pressure rises even more aburptly than it fell, and reaches a level from 10 to 20 mm. of mercury higher than that preceding the induction of the constriction. It then quickly falls again somewhat below, and then gradually rises to, its normal level. When the constriction is gradually

released, the arterial pressure steadily rises, but does not show the peak described when the obstruction is suddenly relieved.

Simultaneously with the fall in arterial pressure, the pressure in the portal vein rises very rapidly to about twice its original value, to fall again precipitately when the obstruction is released and the arterial pressure rises.

It is not always possible to state how completely the hepatic veins are occluded. It is certain that the obstruction was not absolute in all of the experiments. It was undoubtedly complete, however, in many instances, for when the arterial pressure had been reduced by from 37.5 to 42 per cent of its original value, it was not possible to depress it further by increasing the traction and pressure with the rubber tube and finger. It appears to be a justifiable conclusion, therefore, that complete occlusion of the hepatic veins will reduce the systemic blood pressure about 40 per cent.

This does not mean that complete obstruction of the hepatic veins will reduce the total volume of circulating blood only 40 per cent. For with the reduction in volume there is a compensatory vaso-constriction. That the peripheral vessels are markedly constricted during occlusion of the hepatic veins is indicated by the fact that epinephrin, injected during this time, is virtually without effect. When the hepatic veins are quickly released the arterial blood pressure rises very abruptly to a level 10 to 20 mm. of mercury higher than normal. This is probably not due to the excessive quantity of blood that suddenly reaches the heart from the distended liver, but rather to the fact that the left ventricle, now supplied with its normal quantity of blood, delivers it into a greatly reduced total vascular area, that is, into generally constricted vessels. The sudden release of the vaso-constriction when it is no longer needed as a compensating mechanism, is followed by a slight vaso-dilatation, as is indicated by the succeeding depression of arterial pressure somewhat below the normal, and the gradual return to normal.

Immediately upon constricting the hepatic veins the liver increases in size, so much so that in some cases it tended to bulge into the abdominal wound. This occurs while the pressure in the portal vein is rising to at least twice its normal level. Engorgement of the intestines occurs rather slowly and never reaches such apparent proportions as in the case of the liver.

It is also noteworthy that upon obstruction of the outflow of blood from the liver, the systemic blood pressure immediately falls to an irreducible minimum and remains at a constant level for a considerable period (five minutes, the longest in our experiments). The animal does not, therefore, "bleed into the vessels of its splanchnic area." An essential minimum quantity of blood remains in the general circulation and does not accu-

mulate in the huge reservoir behind the constriction. The liver appears to be distended to its maximum capacity, but the mesenteric veins certainly are not. The maximum pressure in the portal vein in these experiments is always far below the minimum pressure in the arteries. Under normal conditions, as pointed out by Macleod and Pearce, the pressures in the branches of the hepatic artery and portal vein must be equal at the point where the two streams unite, that is, at the intrahepatic capillaries. Hence, it seems likely that the greatly increased pressure in the portal vein together with the engorgement of its branches in the interlobular connective tissue may result in a complete cessation of inflow of blood through the hepatic artery. No such mechanism is available to stop the flow into the mesenteric vessels. It might be possible, however, that an extreme degree of vaso-constriction in the splanchnic region occurs as a compensatory mechanism, and thus prevents the accumulation of blood in the intra-abdominal vessels. There is no direct evidence of this. It is suggested, however, by the facts that a general vaso-constriction does occur and that the arterial blood pressures does not continue to fall after constriction of the hepatic veins, as would happen if the animal continued to "bleed into its abdominal vessels."

SUMMARY

1. A method is described by which the hepatic veins can be constricted and even completely occluded, with a minimum of traumatism and of interference with the general circulation.

2. Marked constriction or complete obstruction of the hepatic veins results in a precipitate fall in arterial blood pressure and a simultaneous rise in pressure in the portal vein.

3. Both of these pressures are maintained at a constant level as long as the constriction is kept up. The animal does not, therefore, in this condition, "bleed into its abdominal vessels."

4. On sudden release of the constriction the arterial pressure rises very abruptly to a level 10 to 20 mm. above, then quickly falls slightly below, and slowly rises to, normal.

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STUDIES ON VIGOR

II. The Effect of Castration on Voluntary Activity[1]

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This Department is engaged in a systematic investigation of the relation of various factors to bodily vigor, life span and reproductivity. This report presents certain data secured in one of the series of studies on the influence of endocrine factors on spontaneous activity. The first of the series, dealing with the effect of adrenal deficiency, has recently been published by Durrant (1).

White rats serve as the experimental animals. As an index of vigor, the spontaneous activity is determined by giving the animal access to a revolving cage, one foot in diameter, the revolutions of which are additively recorded by means of Veeder counters. The technic has been described in the paper previously mentioned (1). Further experience has borne out the conclusion that the proportion of the energy of the animal expended in running in the cages is so high in comparison with the total energy expenditures as to serve as a reliable criterion. An exact determination of this proportion would be difficult, perhaps impossible, to secure. We have the impression, however, based on many daily observations, that well above 95 per cent of the activity of normal animals is thus recorded; but in the case of animals of very sedentary trend, such as fat, senile individuals, the proportion is much lower. Indeed, in two instances animals have been kept for a period of weeks in the recording cages without registering more than a half dozen turns a day, though they not infrequently moved from the retiring cage to the revolving cylinder. In such cases, of course, the revolution records would give an exaggerated index of the existing inactivity.

In the experiment herewith reported sixteen castrated male animals and sixteen controls were used. In twelve cases the age of the animals at operation ranged within seventy-one to seventy-seven days. The other four were respectively sixty-two, sixty-eight, eighty-six and ninety days of age. In all but three instances litter mates were used as controls.

[1] Aided by a grant from the "Bache Fund."

Whether, however, this restriction had any other significance than conformity to convention is doubtful.

While our pertinent data, now somewhat extensive, have not been subjected to accurate analysis on this score, the impression is strong that variability of spontaneous activity within litters is quite as great as within the colony as a whole. An endeavor is being made to develop a strain more nearly homozygous for the activity factors. In the meanwhile it has become a routine practice, as in the present study, to "type" the animals as a preliminary to all activity studies, control and experimental animals being matched first as to vigor then as to weight. Several months' experience has shown that in general the performance during the first two weeks in the activity cages foreshadows fairly accurately the vigor subsequently shown.

The results of the castration study are set forth in tables 1 and 2 and in the accompanying figure. After typing the animals, the less vigorous in each case was reserved as control.

At the time the experiments were begun the number of available cages was inadequate and an attempt was made to use each cage for three animals, recording the activity of each for a period of two or three days with periods of four to six days in ordinary housing cages intervening. This plan proved to be not altogether satisfactory, however, and after the first two or three periods, more facilities having become available, the animals were left continuously in the individual cages.

In compiling the tables the total revolutions for ten day periods were divided by the number of days spent in the cages to give the average activity per day. In general, throughout the last hundred days of the experiment each average is based on a full ten days' run. In a few instances, however, owing to escape of an animal from its cage or accidental interference with the smooth operation of the cage, fewer than ten days of activity are included. In all such instances the actual number of days upon which the average is based appears in the table in parenthesis before the figures expressing the number of revolutions per day. During the first two periods the number of days from which the average was calculated varied from two to ten. The fact that the trend of the experiments in which full ten day runs were used throughout is very similar to that of the experiments in which a smaller number of days was included, indicates that no significant error was introduced.

In a few instances attempts were made to influence the activity of the castrated animal by the injection of testicular extracts, by testicle grafts or by thyroid feeding. In no case was there an apparent effect sufficient to invalidate any of the data for purposes of this study. Indeed, in most instances no effects at all could be detected. In table 2 are indicated by the letters a, b and c, respectively, the instances in which a

TABLE 1

Average daily number of revolutions of 16 normal rats tabulated through 12 consecutive periods of 10 days each

ANIMAL	LITTER	AGE	1	2	3	4	5	6	7	8	9	10	11	12	AVERAGE
203	a	54	(4)* 1,640	(3) 2,273	3,812	9,074	10,921	14,159	11,974	9,391	4,587	4,446	5,064	2,276	6,635
115	a	56	2,921	(d) 3,798	3,170	6,708	7,895	12,183	15,936	13,534	13,406	13,757	8,481	5,686	8,956
131	b	52	3,082	(d) 2,614	2,514	5,355	6,502	8,570	10,628	6,177	6,510	6,867	2,662	1,397	5,240
137	c	52		(d) 2,704	1,721	4,487	3,740	2,007	1,762	704	881	2,082	1,185	1,884	(11)‡ 2,105
149	d	55	(3) 2,880	(5) 2,504	(8) 4,006	9,891	14,952	22,829	18,767	(e) 14,940					(8) 11,359
152	d	55	(3) 1,474	(6) 2,350	(8) 4,507	12,474	19,050	19,137	19,929	19,406	10,644	10,460	6,287	6,183	10,992
161	e	54	(2) 1,760	(5) 2,467	(8) 4,180	8,167	8,089	11,711	17,926	19,331	19,041	10,681	4,328	6,031	9,474
162	e	54	(2) 1,221	(5) 2,025	(8) 4,740	9,847	9,178	12,268	16,736	13,068	6,000	5,023	4,309	3,896	7,359
167	f	53	(2) 1,814	(5) 5,855	(6) 5,935	11,943	14,302	15,942	(e) 16,272						(7) 10,295
174	f	53	(4) 2,418	(2) 3,263	(8) 5,284	13,183	16,744	19,003	18,161	(9) 16,628	9,218	6,520	9,545	10,263	10,852
183	g	52	(3) 7,627	6,739	(8) 6,911	13,600	17,592	20,108	(9) 21,255	18,989	12,401	8,602	(9) 13,406	(9) 12,081	13,274
193	h	51	(5) 1,829	(3) 689	(4) 1,355	7,007	12,511	15,527	15,789	12,995	10,291	9,497	6,766	8,578	8,569
218	i	48	(4) 2,754	(2) 3,261	(5) 2,720	6,158	7,990	10,406	16,038	15,314	9,393	8,123	3,278	2,070	7,292
270	j	42	(4) 1,968	(5) 4,115	(4) 3,978	6,850	13,185	15,947	20,691	24,036	12,095	10,721	10,107	12,475	11,347
278	k	66	6,747	3,554	5,244	13,749	13,791	14,056	10,749	7,311	5,121	8,058			(10) 8,838
293	l	70	5,266	3,549	8,869	13,781	11,041	8,090	9,654	14,906	22,632	20,910			(10) 11,870
Average.....		54	(15)† 3,027	3,110	4,309	9,517	11,718	13,871	15,142	(15) 13,782	(14) 10,158	(14) 8,982	(12) 6,285	(12) 6,052	

* In this and all similar cases the number in parenthesis indicates the actual number of days of activity from which the average was determined. Unless otherwise specified 10 days were included.

† In this and all similar cases the number in parenthesis indicates the number of animals the activity of which is averaged. Unless otherwise specified 16 were included.

‡ In this and all similar cases the number in parenthesis indicates the number of ten-day periods averaged. Unless otherwise specified 12 were included.

(d) Sham operation (laparotomy).

(e) Accidentally killed.

TABLE 2

Average daily number of revolutions of 16 castrated rats tabulated through 12 consecutive periods of 10 days each

ANIMAL	LITTER	AGE	1	2	3	4	5	6	7	8	9	10	11	12	AVERAGE
28	x	57	5,583	(4)* 5,185		(9) 2,366	2,120	1,787	2,099	1,429	1,280	1,169	1,424	1,377	(11)‡ 2,347
74	y	51	1,198	3,828	(8) 1,313	(9) 895	1,260	708	1,327	1,233	1,672	761	243	240	1,223
114	a	56	6,716	5,695	4,780	6,146	7,061	5,386	5,944	4,175	3,642	3,532	3,357	2,564	4,916
123	b	52	4,722	3,031	1,743	2,177	1,876	2,206	2,297	(a) 1,879	2,083	2,880	2,166	1,455	2,376
145	c	52		(4) 4,654	7,754	8,743	5,226	7,289	6,014	3,913	5,399	8,231	5,085	4,625	(11) 6,085
148	d	55	(4) 2,092	(4) 4,875	(8) 3,041	5,050	3,286	4,152	4,304	4,256	3,236	(b) 2,908	6,497	7,236	4,244
151	d	55	(4) 2,189	(2) 3,324	(8) 4,408	6,055	5,894	4,585	3,825	(c) 5,037	4,528	5,732	3,790	4,515	4,490
159	e	54	(4) 1,536	(2) 1,743	(7) 1,575	4,732	2,952	1,477	3,091	4,643	2,807	1,909	2,423	2,006	2,574
175	f	53	(2) 1,817	(5) 3,399	(6) 5,203	14,657	13,224	(8) 5,667	8,364	10,303	8,117	7,772	5,012	5,314	7,404
182	g	52	(2) 4,572	(3) 5,861	(5) 1,964	2,578	2,139	2,575	3,100	1,915	1,741	2,133	3,171	3,056	2,900
184	g	52	(2) 1,129	(4) 2,657	(6) 4,190	5,745	4,686	5,188	6,843	5,612	5,405	5,924	4,338	5,192	4,742
197	h	51	(4) 2,001	(2) 2,644	1,600	4,726	4,787	2,864	(c) 3,453	2,525	3,457	2,840	3,179	2,669	3,063
216	i	48	(5) 4,195	(3) 3,915	(9) 2,134	5,542	4,061	1,129	1,364	1,776	2,281	1,855	2,801	2,725	2,815
273	j	42	(2) 1,377	(5) 2,637	(4) 1,960	3,990	5,754	2,693	2,618	2,684	2,125	1,914	1,079	1,435	2,522
280	k	66	(9) 5,025	3,230	1,915	2,702	1,789	1,505	2,599	2,834	2,464	733			(10) 2,479
291	l	70	5,621	3,993	3,351	3,231	4,841	3,413	3,163	3,394	2,285	2,510			(10) 3,580
Average.....		54	(15)† 3,318	3,792	(15) 3,129	4,958	4,435	3,289	3,775	3,600	3,283	3,300	(14) 3,183	(14) 3,172	

*, † and ‡ as in table 1.

(a) Testile graft apparently increased activity.

(b) Thyroid feeding apparently increased activity.

(c) Testicle graft apparently depressed activity.

slight apparent influence was seen. Details of this part of the experiment will be reported later. In the case of animals 123, 151 and 197 testicle grafts, indicated by *a* in the table, apparently depressed the activity for a short time, presumably due to the toxic effect of foreign protein, and in the second period thereafter led to some augmentation of activity.

The charts of the activity of the individual normal animals are in general closely similar to those previously reported by Slonaker (2). A rather striking variability in activity from day to day is a characteristic feature. The cause of the variability is by no means easy to determine. We have studied both the records of individual animals and composite

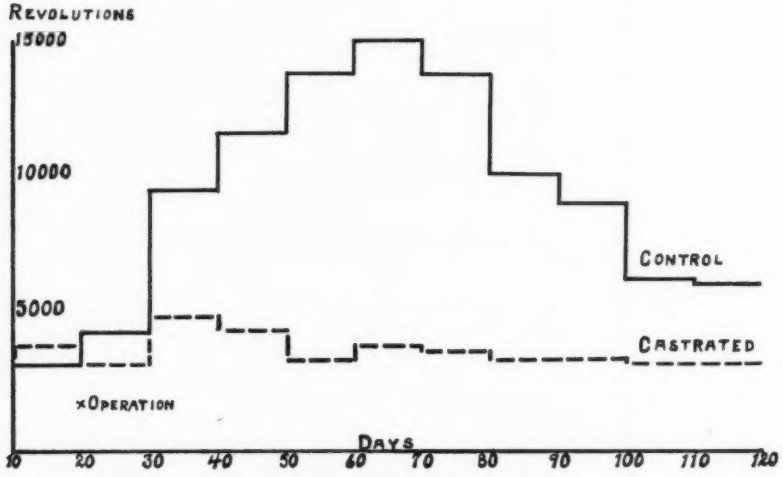

Fig. 1. Activity of normal and castrated rats as manifested by turning revolving cages one foot in diameter.

colony records in connection with such factors as temperature, diurnal variation of temperature, humidity, barometric pressure, wind velocity and direction, and percentage of sunshine, as well as sex, age and season. The diet factor is receiving special study. No simple relationship has been seen between any of the first mentioned factors or any combination of them and daily activity. Further studies of the operation of these factors are in progress. Suffice to say at this point that the incidental daily variations were not sufficient to obscure the trend of the experiments.

In only one instance did the activity curve of an experimental animal fail to drop strikingly below that of the control. In this instance the control animal, no. 137, was obviously abnormal from the fourth period on-

ward, its activity falling to perhaps a fourth of what would be predicted from its earlier performance. Since, however, no cause for the lethargy could be detected it was left in the series.

The results of the experiment are sufficiently obvious from the accompanying figure in which are given the average daily revolutions through ten day periods. In most cases the observations were continued for one hundred and twenty days, i.e., for one hundred days after the operation. In only one respect do the graphs seem to demand comment. The depression appearing in the third period is due partially to the effect of operative trauma rather than castration, per se. The height of the composite graph of the control animals is also slightly depressed below a true normal because of the fact that sham operations were performed in three cases. These operations, as a matter of fact, were laparotomies and distinctly more severe than simple castration. In addition to the depression ascribable to trauma is that due to the anesthetic, amytal, which was used in most of these operations. This substance, a barbituric acid derivative, would be expected to give a fairly prolonged depressant effect. From a study of the animals here reported and various others subjected to sham operations it early became apparent that such control operations are unnecessary. The effect of trauma, per se, is relatively insignificant and short lasting. Our experience serves to confirm the conclusions of Macht and Seago (3), who found that the maze performance of rats was practically unaffected by simple laparotomies. Incidentally, one of the most satisfactory characteristics of the white rat for this type of experimental work is its relative immunity to traumatic shock and ordinary pyogenic infections.

Incidence of castration depression. Assuming that the greater activity of the control animals was due to the activity of a testicular hormone, the question arises: How long does this hormone persist after castration? The problem was studied by superposing the activity graphs of the individual experimental and control animals and noting the point at which they began to diverge. In most instances this point was so sharply marked that it could be fixed definitely as occurring on a given day. In two instances the divergence of the curves began as early as the fifth day. The longest period before the effect of castration was apparent was twenty-six days. As nearly as could be determined from so small a number of animals the mode was twelve days.

Another problem is: How early does the testicular hormone begin to function? As Slonaker has pointed out, the activity begins to increase fairly rapidly with the onset of puberty. In our experience it reaches the highest point at four or five months. The "control" graph in the accompanying figure shows clearly the course of the activity through the three most active months. After the fifth month the activity ordinarily slowly

declines to a sustained low level of a few hundred revolutions a day. In case of the few senile animals we have had opportunity to study, this has ordinarily ranged from one hundred to six hundred revolutions a day, though in two instances in which the animals were senile when placed in the cages, practically no activity was registered over a considerable period.

The total activity of the different animals throughout the four months of the experiment varied within fairly wide limits. The most active rat in the control series, no. 183, made 1,592,880 revolutions (approximately 950 miles) and of the castrated group no. 175 made 888,480 revolutions. Excluding rat 137 which, as previously mentioned, was abnormally sluggish, the least active control rat made 628,800 and the least active castrate, 146,760 revolutions.

The foregoing data represent merely a quantitative treatment of a phenomenon long familiar in a qualitative way. Speculation as to the underlying reason for the depression observed in castrated animals would seem at this juncture rather unprofitable. The literature on the topic is as extensive as it is unconvincing. The situation seems now to demand further investigation rather than another threshing over of the much belabored straw.

SUMMARY

Sixteen control and sixteen castrated white rats were studied by means of automatically recording revolving cages. The animals were castrated at about seventy days of age after a two weeks' preliminary study. Beginning at about the twelfth day on the average the castrated animals began to lag behind the controls. At the fiftieth day after operation the average daily activity of the control group was at its highest point, namely, 15,142 revolutions (approximately 48,000 feet) while the average of the castrated group was 3,283 revolutions. At the end of 100 days after operation the average records were respectively 6,052 and 3,172 revolutions daily. The highest total activity for the entire period for an individual control animal was 1,592,880 revolutions (approximately 950 miles) and for a castrated, 888,480 revolutions.

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THE PRESENCE OF SECRETIN IN THE INTESTINAL JUICE

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Bayliss and Starling (1), (2) showed that hydrochloric acid extraction of the mucous membrane of the upper part of the small intestine, yields a specific chemical excitor of the pancreatic secretion. They called this substance "secretin." Popielsky (3) and his followers, and recently Luckhardt, Henn and Palmer (4), have disputed both the specificity of secretin formation in the mucous membrane of the small intestine and the specificity of the secretin effect on the pancreas. Nevertheless the observations of Bayliss and Starling must be regarded as generally sound on the very ground of varied and exhaustive tests undertaken by them as well as by Wertheimer (5), Camus (6), Fleig (7), Dixon and Hamill (8), Lalou (9), Edgar Zunz (10), Divry (11), Derouaux (12) and others. All of the latter group of investigators accept secretin as formed in the upper part of the small intestine whence it enters directly into the blood stream after the absorption of the acid contents of the intestine.

There is also evidence that secretin may not only enter into the blood, but that it can also be found in the lumen of the small intestine. Wertheimer and Lepage (5) in 1902 established the fact that hydrochloric acid which has been in the small intestine for some time, possesses a distinct secretin effect. Wertheimer and Duvellier (5) convinced themselves of this fact again in 1910. Their experiments were repeated by I. Matsuo (13), who fully confirmed the fact that hydrochloric acid gains the secretin effect during its stay in the small intestine. Matsuo made a thorough study of this condition and brought to light some interesting details. He has made systematic use of this process as a secretin-generating method.

None of the above writers, however, have raised the question as to the way in which the hydrochloric acid acts under the circumstances. In 1902 L. Camus suggested the idea that secretin might be present in the intestinal juice, but he did not put it to experimental test.

Approaching this question as to the manner in which the acid contents of the intestine gets its secretin effect I have tried to ascertain experimentally whether the intestinal juice itself contained any quantity of secretin. For this purpose I collected the intestinal juice of dogs with permanent Thiry-Vella fistulae. This juice was subsequently worked up

for secretin according to the method of Bayliss and Starling (boiling in 0.4 per cent hydrochloric acid, neutralizing with 10 per cent NaOH and filtering). In order to try the effect of this intestinal juice preparation on the pancreas, it was introduced intravenously in dogs with permanent pancreatic fistulae or tested in acute experiments. The result of all experiments was, that *the intestinal juice obtained from a Thiry-Vella fistula of the upper part of the small intestine invariably contained secretin in rather considerable quantities.*

Below I submit the records of experiments in which the effect of the common secretin preparation (from the duodenal and jejunal mucous membrane) can be compared with that of the intestinal juice secretin.

I have varied these experiments in some respects. The intestinal juice was collected from the intestinal fistula during periodic activity of the alimentary canal (hunger contractions), after mechanical irritation by the introduction of a drainage tube or after an injection of calomel. The dogs with fistulae for obtaining the intestinal juice were experimented on in some cases immediately after feeding and in others before the feeding or even after a prolonged hunger (from 12 to 60 hours after the last feeding).

The result of the experiments, however, always had the same meaning; in all cases a clearly expressed secretin effect followed. The strongest secretin effect was always shown by the preparations of the periodic intestinal juice.

In studying the other digestive juices I tested them for the presence of secretin by working them up in exactly the same manner as used for the intestinal fluid. Saliva, bile and pancreatic juices showed no secretin effect, but the gastric juice both from the fundus and pylorus pouches showed some effect, which was always rather weak, though never absent.

If the intestinal juice has been collected from a Thiry-Vella fistula the flaky part of the juice shortly settles forming a thick sediment. Thus it was easy to investigate separately the sediment and the supernatant fluid. Below are given a few records of experiments conducted in this way.

As will be seen, secretin is present only in the sediment of the intestinal juice, and not in the liquid part. Having become satisfied of this fact, I thereafter used only the sediment in making preparations for test. In order to obtain the sediment quickly and completely the collected material was at once centrifuged for half an hour, the almost clear fluid on top being discarded. A definite quantity by weight of the sediment was then mixed with a tenfold volume of 0.5 per cent of hydrochloric acid and further worked up for secretin in the usual way. The tenfold dilution was chosen because more concentrated solutions did not seem to possess any stronger secretin effect. From the preparations obtained in this way I gained the conviction that the sediment of the periodic intestinal juice showed the greatest amount of secretin.

TABLE 1

In the acute experiments a cannula was introduced into the large pancreatic duct, the small one being tied. The pylorus was separated from the duodenum by a ligature. The cannula was connected with a small calibrated glass tube. Each figure of the table shows the number of divisions on the glass tube by which the pancreatic juice moved on any minute. Immobilisation in all of these experiments was secured by spinal transection just below the medulla. The time of secretin injection and the origin of the preparation are specifically stated for each experiment. The secretin injections were made intravenously.

TIME IN MINUTES	EXPERIMENT 5, 10/3, 1922		EXPERIMENT 10, 12/1, 1923		EXPERIMENT 15, 16/3, 1923		EXPERIMENT 23, 24/8, 1923		EXPERIMENT 24, 30/8, 1923	
1	9	11			0	0	1	1		
1	11	6			0	0	1	1	3	1
1	6	7	3	4	0	0	1	2	2	1
1	7	4	1	1	0	0	1	2	2	1
1	5	4	2	4	0	0	1	2	1	1
1	4	2	3	2	0	0	2	2	1	1
	15.0 cc. secretin from *mucous membrane* of the duodenum-jejunum	15.0 cc. secretin from *sediment* of the *intestinal juice*	10.0 cc. secretin from *mucous membrane* of the intestine	10.0 cc. secretin from *sediment* of the *intestinal juice*	10.0 cc. secretin from *mucous membrane* of the duodenum and jejunum	10.0 cc. secretin from sediment of the *intestinal juice*	4.0 cc. secretin from *mucous membrane* of the duodenum and jejunum	4.0 cc. secretin from sediment of the *intestinal juice*	6.0 cc. secretin from *mucous membrane* of the duodenum and jejunum	6.0 cc. secretin from sediment of the *intestinal juice*
1	3	14	6	0	7	6	13	10	3	3
1	30	29	25	8	68	78	65	53	40	67
1	255	118	135	45	135	127	40	75	53	93
1	245	96	117	60	135	128	30	75	35	80
1	214	80	73	65	105	112	18	60	23	70
1	221	131	57	58	135	115	10	40	13	52
1	165	124	46	55	95	101	24	30	15	32
1	170	114	42	47	130	94	12	20	11	23
1	165	102	27	40	75	90	5	15	7	15
1	170	99	35	36	95	160	6	5	7	13
1	150	88	32	33	85	85	6	5	4	12
1	140	75	26	31	120	70	5	9	2	7
1	140	70	23	27	55	65	6	6	1	9
1	130	60	20	21	65		5	3	3	6
1			18	21	60	45	3	3	4	7

It was interesting to investigate whether the secretin in the intestinal juice was associated with the "depressor substance" of Bayliss and Starling and extracted together with it, as is the case in preparations of the mucous membrane. Experiments undertaken with this purpose in view, in which I observed the pancreatic secretion and recorded the blood pressure curves simultaneously, showed that in the sediment of the intestinal juice one may easily obtain a secretin preparation, which stimulated pancreatic

TABLE 2

For explanations see table 1.

TIME	EXPERIMENTS WITH SECRETIN FROM OTHER DIGESTIVE JUICES			
	Experiment 5, 19/3, 1922	Experiment 3, 4/3, 1922		Experiment 8, 15/1, 1922
1	32	6	9	10
1	25	9	14	7
1	25	8	7	0
1	15	6	13	0
	20 cc. secretin from the bile	20.0 cc. secretin from the pancreatic juice	20.0 cc. secretin from the gastric juice of the fundus	20.0 cc. secretin from the pylorus juice
1	17	8	14	0
1	13	6	7	0
1	13	7	16	1
1	13	5	21	5
1	15	5	15	15
1	15	7	20	7
1	20	7	21	3
1	22	7	20	2
1	23	6	12	7
1	50	4	17	2
1	25	5	11	1
1	25	4	13	
1	25	8	11	
1	25	4	15	
1	21	4	9	

secretion strongly but had no effect whatever on the blood pressure. The only point of importance in making the preparations was to use great care in neutralizing the boiling extract. As the intestinal juice preparations did not require special care to remove the "depressor substance" I concluded that the secretin obtained from the intestinal juice was purer than that from the mucous membrane, i.e., without any admixture of the "depressor substance." The secretin obtained from the intestinal juice practically corresponds to that which Bayliss and Starling (1) obtained from the "desquamated epithelium."

Another proof has thus been adduced that secretin is a totally different substance from the "depressor substance" of Bayliss and Starling or from the "vasodilatin" of Popielsky, and that the effect which it produces is in a large measure independent of blood pressure fluctuations. I do not show any blood pressure curves, but the experiments have been repeated several times and do not allow any doubt on this point. A demonstration with simultaneous observation of the pancreatic secretion and of the blood pressure was given in January, 1923, at a meeting of the Physiological Conference of Petrograd, and those present could convince themselves

TABLE 3

For explanations see table 1.

TIME IN MINUTES	EXPERIMENT 4, 7/3, 1922		EXPERIMENT 10, 12/1, 1922		EXPERIMENT 11, 27/2, 1923	
1	3	2	4	7	2	5
1	3	1	7	5	1	3
1	3	2	3	7	1	5
1	4	1	3	8	1	3
	20.0 cc. secretin from the sediment of the intestinal juice	20.0 cc. secretin from the liquid part of the intestinal juice	9.0 cc. secretin from the sediment of the intestinal juice	10.0 cc. secretin from the liquid part of the intestinal juice	10.0 cc. secretin from the sediment of the intestinal juice	10.0 cc. secretin from the liquid part of the intestinal juice
1	5	2	3	6	4	2
1	5	2	12	3	5	2
1	41	2	35	8	23	2
1	65	2	84	4	40	1
1	54	2	55	6	57	1
1	44	2	55	3	55	1
1	35	2	47	3	51	1
1	23	3	35	4	44	1
1	20	4	53	4	35	1
1	8	3	38	6	30	1
1	8	3	32	4	22	2
1	8	3	35	2	24	1
1	7	5	30	5	16	0
1	4	5	18	2	17	1
1	5	2	13	4	12	0

both of the strong secretory effect as well as the complete absence of a depressing effect induced by preparations made from the intestinal juice.

In conclusion it should be mentioned that I have done some experiments in which I prepared the secretin from the sediment of the intestinal juice not with hydrochloric acid solutions, but by boiling it with physiological saline. In three cases I obtained preparations which in their effect approached in strength the secretin prepared with hydrochloric acid.

Therefore, Carlson's (14) opinion about the "gastrins," that they "are artefacts developed in the decomposition of the foods, or in the extraction of the mucosa and do not represent physiological mechanism" may hardly

be applied to the "secretin." It seems more accurate to look upon the pancreatic "secretin" as a normal product always present in the epithelial cells of the mucous membrane of the upper part of the small intestine.

In a subsequent paper I intend to discuss the action of secretin obtained from the intestinal juice of other digestive glands.

My thanks are due to Prof. I. P. Pavlov for his advice and criticism during the performance of this work.

SUMMARY

1. Intestinal juice collected from Thiry-Vella fistulae always contains secretin in its sediment.

2. From other digestive juices—saliva, bile and pancreatic juice—no secretin was obtainable. In the gastric juice both of the fundus and the pylorus part a small quantity of secretin is always to be found.

3. By careful neutralization of the acid used for extraction whilst boiling one can obtain secretin preparations which have no depressing effect on the blood pressure (i.e., without the "depressor substance" of Bayliss and Starling or "vasodilatin" of Popielsky).

4. By boiling with physiological saline solution one can also extract secretin from the intestinal juice sediment.

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STUDIES ON EPILEPSY

II. The Occurrence of Clonic Convulsive Seizures in Animals Deprived of the Cerebral Motor Cortex[1]

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The belief that, in a typical convulsive attack, the clonic element is due to the motor cortex, while the tonic phase is essentially subcortical, is generally accepted. Much clinical, pathological and experimental evidence has been adduced to support this viewpoint. The fact of the sensitiveness of the motor cortex to mechanical and chemical irritants and to the electric current, the frequency of clonic convulsions in irritation of the Rolandic area by foreign bodies, cicatrices, blood clot, and by superficial tumors seemed valid proof that the cells of the Rolandic area were responsible for the motor perturbations of a clonic character. Even when a disease entirely beneath the cortex was found to exist, the convulsive seizures were considered to be due to an effect upon the cortex of the more deeply situated disease.

The investigations made many years ago by Franck and Pitres, Munk, Schaefer, Bechterew and others seemed to make it certain that the clonic phases of a convulsion occurred only when the motor cortex was intact and that in animals after removal of the cruciate area the muscular contractions that were produced by appropriate stimulations were purely tonic in character. Some investigators cast doubt upon the correctness of these conclusions, but the view that clonic seizures are due to stimulation of and discharge of motor impulses from the cortex, and tonic seizures from deeper subcortical areas has gradually prevailed.

We have again taken up this question and have subjected it to a careful experimental and critical analysis.

A large number of experiments were done upon young and adult cats, in which convulsions were produced by the injection of a convulsing agent after smaller or larger areas of the brain had been removed.

[1] This study was made possible through a grant for a research on epilepsy from the Commonwealth Fund of New York to the New York Neurological Institute, for which we desire to express our gratitude.

As a convulsing agent we used a solution of absinth, of which stated amounts were injected intravenously. The amount of the drug that would cause a convulsion in a normal animal and the character of the convulsions, was described in a previous paper.[2] As stated in that publication, the smallest quantity of absinth solution, per pound of animal, that would cause a typical convulsion, we called a "normal convulsing dose." Furthermore we presented the evidence that a "normal convulsing dose" of absinth, injected intravenously, exerts its effect upon the cerebral hemispheres. When the blood vessels to the animal's head were clamped off so that no absinth could reach the brain, no convulsions were every produced by the injection. Other investigators who used absinth had injected much larger amounts and had produced clonic and tonic convulsive seizures after section of the brain stem and even of the spinal cord just below the medulla. For these reasons the experiments made many years ago by Gotch and Horsley, Pasternatsky and Magnan, led them to believe that absinth convulsions are not due to the brain alone, but can start from any part of the central nervous system and, as just mentioned, some recent workers have arrived at the same conclusion.

After the removal of parts of the cerebral hemispheres, the results of the injection of minimum doses of the convulsing agent will vary with the period of time that is permitted to elapse between the operation and the absinth injection. As was to be expected, the susceptibility of the brain to stimulation is diminished for a number of hours and days after a cerebral trauma. This probably partly explains the fact that, except in the so-called status epilepticus, there are longer or shorter intervals between convulsive attacks in so-called epilepsy. That there may be some connection between the thyroid (and parathyroid and perhaps other glandular structures) and the susceptibility of the brain to respond to irritation by the production of convulsive seizures, was pointed out in our first paper.

We have given the absinth injections not only early, after the animal had recovered from the anesthesia used during the cerebral operation, but also one to three weeks later, after the animal had entirely recovered from the craniotomy.

The following is a summary of the main results of the investigation—each group of conclusions being illustrated by abbreviated protocols of a few of many experiments.

1. Excision of one motor cortex. One to two hours after the animal had recovered from the ether anesthesia, a "normal convulsing dose" of absinth solution (0.03 to 0.04 cc. per pound of animal) was injected into the femoral vein.

[2] Elsberg and Stookey: Arch. Neurol. and Psychol., 1923, ix, 613.

Within fifteen seconds to two minutes after the injection, typical clonic and tonic convulsive seizures occurred on the same side of the body as that on which the motor cortex had been excised. On the opposite side, however, the limbs remained in tonic extension for the period during which the convulsive movements were occurring on the other side.

Experiment 332. Young cat, weight 3½ pounds

TIME		RESULT
3:00 p.m.	Excision of three-quarters of left motor area under ether anesthesia.	
4:47 p.m.	0.14 cc. (0.04 cc. per pound) of absinth injected into femoral vein.	Convulsive twitches of limbs of left side
4:54 p.m.	0.14 cc. (0.04 cc. per pound) of absinth injected into femoral vein.	Severe convulsion of left side of body. Right limbs stiff in extension. The limbs of the right side remained stiff for several minutes after the convulsive movements of the left side had ceased
5:05 p.m.	0.56 cc. (0.16 cc. per pound) of absinth into vein.	Severe convulsion limited to left side
5:08 p.m.		Animal died

Post-mortem examination and study of the brain after hardening in formalin, showed that practically the entire left motor cortex had been removed.

If, however, after excision of one motor cortex, several weeks were allowed to elapse before the absinth injections were made, the effect was a different one. The injection was promptly followed by tonic and clonic convulsive seizures, which affected the limbs of both sides but

Experiment 346. Cat, 5 pounds

Excision of left motor area. Injections of absinth three weeks later.

TIME		RESULT
4:48 p.m.	Injection of 0.2 cc. (0.04 cc. per pound)	Convulsive twitchings of all limbs, more marked on the left
5:07 p.m.	Injection of 0.3 cc. (0.06 cc. per pound)	Convulsion of all four limbs for one minute, with twitchings of both ears. Although the convulsions seemed of equal severity on the two sides, the animal constantly rolled to the right during the convulsion
5:12 p.m.		Animal died

Examination of fresh and of hardened brain showed that the left motor area had been completely removed.

were most violent on the side still under cortical control. In many of the experiments, the tonic extensor phases of the convulsions were more distinct in the limbs that had been deprived of cortical control (by excision of the motor cortex), and for a short period after the convulsive attack was ended there was some extensor spasm in the limbs of that side.

Experiment 353. Adult cat, 6 pounds

Left motor area and left frontal lobe removed. One week later, injections of absinth.

TIME		RESULT
4:20 p.m.	Injection of 0.24 cc. (0.04 cc. per pound)	Tonic and clonic convulsions of both sides of body but more extensor spasm on the right
4:36 p.m.	Injection of 0.48 cc. (0.08 cc. per pound)	Convulsive twitches of all four limbs
4:48 p.m.	Injection of 0.72 cc. (0.12 cc. per pound)	Convulsive twitchings, more pronounced on the left, so that the animal rolled over to the right; considerable extensor rigidity in fore limbs
4:55 p.m.	Animal killed with chloroform	

Post-mortem examination of fresh and of hardened brain showed that there was a complete removal of the anterior part of the left frontal lobe, which extended about 5 millimeters back of the location of the cruciate sulcus. There was also a slight injury of the right hemisphere on its median surface.

2. Excision of both motor areas. In a large number of cats, both motor areas and the frontal lobes were excised and the injections of absinth then given. In our early experiments, the operation was completed at one sitting, but the mortality was very high because of the helplessness of the animals after the operations. To obviate this, the operations were divided into two stages, one week apart. One side of the brain was first excised and the animal allowed to recover for one week before the motor area of the other side was removed.

The results that were obtained in this series were constant, if those experiments were excluded in which the autopsy showed that the excision of both motor areas had not been complete.

After excision of both motor areas, intravenous injections of absinth caused typical convulsive seizures with both tonic and clonic phases. These convulsions did not differ essentially from absinth convulsions that occur in unoperated animals except that some of the animals seemed less susceptible to the absinth so that slightly larger doses had sometimes to be given.

A few protocols follow:

Experiment 343. Cat 5¼ pounds

Injections of absinth one week after excision of both motor areas.

TIME		RESULT
12:55 p.m.	0.21 cc. (0.04 cc. per pound) of absinth injected	Increased salivation and few clonic twitches of both ears
1:02 p.m.	0.26 cc. (0.04 cc. per pound) of absinth injected	Typical clonic spasms of all limbs
1:17 p.m.		Death after three subsequent injections of absinth which did not produce any convulsions

Examination of the fixed brain showed that the destruction of the left motor area was complete but that grossly on the mesial aspect of the right hemisphere, some of the motor area remained. The destruction of both sides was, however, probably complete.

Experiment 352. Cat 4 pounds

February 14, 1924: Excision of left motor area.
February 22, 1924: Excision of right motor area.
Injections of absinth one week later

TIME		RESULT
11:30 a.m.	Injection of 0.16 cc. (0.04 cc. per pound) of absinth	Very slight twitches of limbs
11:50 a.m.	Injection of 0.1 cc. (0.025 cc. per pound) of absinth	Few twitches of muscles of head
12:00 m.	Injection of 0.2 cc. (0.05 cc. per pound) of absinth	Marked clonic spasms of limbs of both sides
12:14 p.m.		Death

Examination of the hardened brain shows that the motor areas of both sides had been destroyed. The lesion was more extensive on the left side where hemorrhage and softening extend clear to the ventral surface of the hemisphere.

Experiment 355. Cat, 6 pounds

Injections of absinth one week after excision of both motor areas with Pacquelin cautery.

TIME		RESULT
2:35 p.m.	Injection of 0.24 cc. (0.04 cc. per pound) of absinth	Typical clonic convulsions of all limbs
3:35 p.m.	Injection of 0.36 cc. (0.06 cc. per pound) of absinth	Clonic twitches of all limbs followed by tonic and clonic convulsions and twitches of all limbs for three minutes
4:10 p.m.	Injection of 0.48 cc. (0.08 cc. per pound) of absinth	Clonic twitchings of limbs
4:35 p.m.	Injection of 0.6 cc. (0.1 cc. per pound) of absinth	Clonic twitchings of limbs
4:37 p.m.		Death from respiratory failure

Examination of the hardened brain showed that the cruciate sulcus of both sides had been destroyed.

3. In this series of experiments, sections of the brain were made above the optic thalami, in front of the corpora quadrigemina, between the corpora quadrigemina, and through the posterior quadrigeminal bodies. We made no attempt to keep the animals alive for a period after the operation, so that the absinth injections were given soon after the animals had recovered from the anesthesia.

In all of these experiments, the extensor rigidity caused by the operation was markedly increased after each absinth injection. In a few instances, slight clonic twitches of the muscles were observed, but these were rare and ill-defined.

CONCLUSIONS

These experiments show that both tonic and clonic convulsions of the limbs can be produced in cats by absinth after the excision of one or even of both motor areas, if an interval sufficient for the return of good locomotor reactions is allowed to elapse after the experimental operation. When only one motor area has been destroyed, there may be more tonic extensor spasm on the side which has been deprived of cortical influences, and slight extensor rigidity may be noted for a short period after the convulsive phenomena have ceased. The greater susceptibility of the uninjured hemisphere to absinth is shown by the rolling of the animal to the side of the uninjured hemisphere during the absinth convulsions.

The motor cortex is unnecessary for those motor stimuli which produce the recurrent muscular contractions that occur in a typical convulsive attack due to absinth.

DECEPTIVE EFFECTS OF EXTRACTS OF SUPRARENAL CORTEX

Cardiac Effect Produced by Potassium Content; Intestinal Effects due to Epinephrin and Choline

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Alcoholic extracts of the suprarenal cortex,[1] when perfused through the frog's heart, produce very striking effects; viz., partial to complete heart block (fig. 1), which is not influenced by atropin, but which disappears promptly when the preparation is washed.

Before accepting this effect as characteristic of a special constituent, it was of course deemed necessary to inquire whether it could be due to any of the known constituents of suprarenal cortex; especially to choline-like substances, perhaps modified by the presence of epinephrin; or to the potassium which is present in all tissue extracts.

The fact that the action was not influenced by atropin suggested that it was not due to choline or its esters; comparative experiments confirmed the differences; for the cholines depress the contractility of the heart without block; and they present a complete mutual antagonism with atropin. The possibility that the choline effects might be modified by the presence of some epinephrin was also excluded, for experiments showed that epinephrin merely antagonizes the choline; i.e., it restores the contractility, without any tendency whatever toward heart-block. Nor did the destruction of the last traces of epinephrin in the extracts, by preceding oxidation, modify the heart-block response (fig. 1).

Potassium salts are known to produce heart-block. The possibility whether these might be responsible for the phenomenon was tested by incinerating the extract, and perfusing solutions of the ash. This produced results identical with those of the extract itself, i.e., partial and complete block (fig. 2). The ash required a higher concentration, it is true; for instance, in one experiment the concentration of the ashed extract had to be raised to double that of the unashed extract; but such quantitative differences would be expected from the alterations that the mineral

[1] See appendix for the methods of experimentation.

Fig. 1. Adrenal cortex on atropinized perfused frog heart: The heart was atropinized at the first arrow (4 drops of 1 per cent atropin, through standpipe). At the second arrow a Ringer's solution of the oxidized cortical extract, corresponding to 10 per cent of moist gland, was substituted for the plain Ringer's. A few minutes later a progressive heart-block develops (note the different rate of the small auricular and the strong ventricular contractions). This culminates in complete arrest. At ↑↑, the perfusion of normal Ringer's solution is resumed, and the heart-block disappears at once.

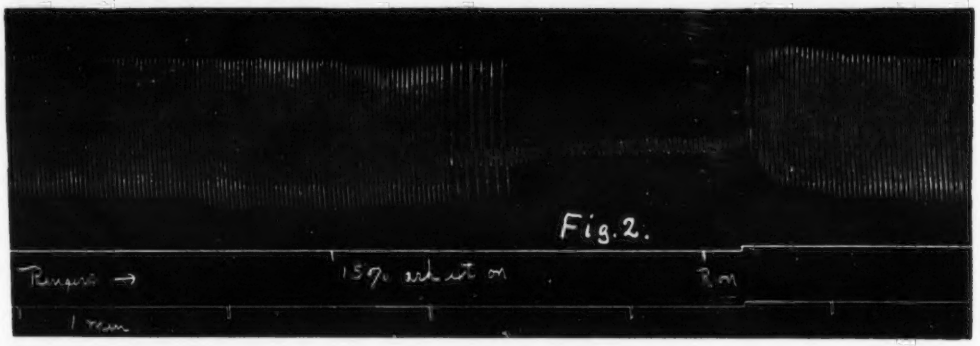

Fig. 2. Incinerated cortical extract on perfused frog heart: An extract of the ash corresponding to 15 per cent of the fresh gland in Ringer's solution is perfused. The phenomena corresponded exactly to those of the original extract, as shown in figure 1.

constituents undergo by incineration. It is therefore very probable that the effect is due entirely to the mineral constituents; and of these, potassium alone produces heart block. Direct comparison showed that effects identical with those of the extract and of the ash are produced by the addition of potassium to the Ringer's perfusion solution.

There remained therefore no doubt that the striking cardiac effects of the extracts of suprarenal cortex were due to the potassium that was extracted from the tissues.

Experiments on strips from the excised hearts of turtles furnished interesting confirmation of this fact. The extracts were found to act much more powerfully on ventricular strips than on those from the auricle. The ventricular contractions were greatly slowed and soon arrested; the auricular contractions were under the same conditions only slightly slowed and weakened, never arrested. These phenomena disappear promptly if the preparations are washed. The effects of the extracts were duplicated by solutions of their ash, and by potassium salts. The relative insusceptibility of the auricular preparations is also characteristic of potassium.

A problem of a different kind is presented by the action of these extracts on *the excised intestine*. The untreated extracts produced a prompt inhibition of Magnus preparations, i.e., an effect characteristic of epinephrin. If the epinephrin is destroyed by oxidation with hydrogen peroxide, the extracts stimulate the intestinal movements, similar to a weak choline solution; and this effect is definitely antagonized by atropin. The intestinal action may therefore be attributed to the choline or related substances that exist in the suprarenal cortex.

CONCLUSIONS

Alcoholic extracts of the suprarenal cortex produce a striking heart-block when perfused through frogs' hearts. This is not antagonized by atropin, and is due to the potassium extracted from the tissue.

They differ entirely from the effects of choline or acetylcholine. These latter weaken the cardiac contractions to the point of suppression, but without heart-block. This effect is removable by adequate doses of atropin or of epinephrin, the antagonistic phenomena being apparently identical for both; except that the epinephrin effect may be pushed to actual stimulation, whilst atropin goes only to the removal of the choline-depression.

The intestinal movements are depressed by the extracts; this is due to traces of epinephrin; and when these are removed by oxidation, a moderate choline-stimulation becomes apparent. This is removable by atropin.

APPENDIX: METHODS OF EXPERIMENTATION: *1. The preparation of the extracts:* The adrenal glands were obtained within 15 minutes after the death of the animals. The renal fat adhering to the tissue was trimmed off

those of the original extract, as shown in figure 1.

and the glands quartered or halved with a sharp knife. The medullary tissue, which in color and consistency is in sharp contrast with that of the cortex, was scraped off with the knife. The gross presence of medullary tissue is easily discernible macroscopically, but to insure its complete removal a small portion of the internal surface of the cortical tissue was also scraped off. The remaining tissue weighing 350 grams was then cut into small pieces and placed in two volumes of 0.2 per cent HCl in 95 per cent ethyl alcohol.

The extraction was continued at room temperature for 24 hours. The menstruum was then poured off and the tissue expressed for its fluid content. The entire volume of fluid thus obtained was filtered twice, neutralized to litmus with Na_2CO_3 and evaporated in a warm air current or on a water bath. The residue remaining was extracted for 15 minutes with warm Ringer's solution or 0.7 per cent NaCl, equivalent in volume to the original wet weight of the tissue extracted. The resulting solution was filtered and used for testing.

2. Oxidation of epinephrin: This was accomplished by adding hydrogen peroxide solution during the evaporation of the alcoholic extracts. The resulting preparations were then tested on a segment of rabbit's intestine. Either epinephrin or traces of H_2O_2 were easily noted through this procedure; and if these were still present their oxidation was completed by re-evaporating. Hydrogen peroxide solutions usually contain acetanilid as a preservative; but only small volumes of H_2O_2 were used so that the acetanilid content of the final solution was never higher than 0.001 to 0.003 per cent. It could therefore be disregarded.

3. Ashed extracts: Portions of the original alcoholic extracts were evaporated and the residue placed in a crucible and completely ashed. The final ash was taken up in that volume of distilled water, Ringer's solution or 0.7 per cent saline which was equal to the wet weight of the cortical tissue represented by the evaporated extract.

4. Frog heart perfusions: The fluids were perfused from aerated perfusion bottles into the sinus venosus by means of a two way cannula equipped with a pressure standpipe. Two centimeters of water pressure was used throughout the experiments. The tracings were taken from the ventricular apex.

5. Turtle heart strips: Auricular and ventricular strips were attached to the foot of a suitable holder and to heart levers. The strips were then immersed in a known volume of Ringer's solution and 0.7 per cent saline respectively. Drugs were added directly to the bath solution.

6. Rabbit intestinal segment: The segments were excised from a rabbit under urethane anesthesia, mounted for recording longitudinal contractions only, and immersed in aerated Locke's solution at 38°C. Drugs were added as in the turtle-heart experiments.

THE ACTION OF ACIDS ON CELL DIVISION WITH REFERENCE TO PERMEABILITY TO ANIONS

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In a previous communication by Smith and Clowes (1924 a) it was shown that the inhibitory action of carbonic acid on the segmentation rate of *Asterias* and *Arbacia* eggs is influenced in the direction and degree to be expected if the carbonate ions in the external fluid participate in the determination of the acid-base equilibria in the cell. It was tentatively proposed to consider these cells as permeable to both CO_2 and HCO^-_3 ions, and under this proposition it was shown that the observed equilibria in various carbonate-carbonic acid mixtures which just repressed cell division were such as to establish the same intracellular hydrogen-ion concentration.

It seemed to the present author that if this proposition were correct it should be possible to confirm it by similar observations on the other acids which penetrate these cells, for by its terms the intracellular hydrogen-ion concentration necessary to repress cell division should be the same regardless of the nature of the acid used. Furthermore, it appeared possible to show by the same method whether a similar condition of apparent permeability existed for anions other than HCO^-_3.

Since the pioneer investigations of de Vries, Pfeffer and Overton on the semipermeability of living protoplasm, numerous attempts have been made to determine directly by the use of natural or applied indicators the physical and chemical factors that control the penetration of acids into living cells. Harvey (1914) immersed cells in simple 0.01 N solutions of various acids, and was able to establish a significant difference in the penetrating powers of the organic acids and the mineral acids, and a slight degree of correlation in the former group between penetrating power and physical properties. Crozier (1916–22) used acid solutions varying from 0.1 to 0.002 N made up in pure water; Haas (1916) used solutions 0.01 N either with respect to the acid or to the H^+ ion; Collett (1919, 1920a) used solutions varying from 0.01 to 0.001 N and also solutions at pH 3.5 to 4.75; at another time Collett (1921b) examined the antagonism between simple acid solutions and NaCl and $CaCl_2$; Brooks (1923a), in order to have comparable conditions, added sufficient quantities of various acids to sea water to bring the resulting mixtures to pH 3.6. From other experiments Brooks

[1] Fellow in the Medical Sciences, National Research Council.

(1923b, c) gives evidence of the penetration of HCO_3^- and Cl^- anions from solutions containing CO_2 and ammonia. Apart from a general confirmation of Harvey's observations, these investigators have reached no common agreement on the factors determining the rate of penetration of acids.

Some doubt may be entertained about the maintenance of physiological integrity in tissues transferred from their normal baths of either balanced and approximately neutral salt solutions, or casual water, as the case may be, to solutions of negligible salt content and excessive acidity. And in the absence of control and of examination of the important physico-chemical variables it remains impossible to determine to what extent the results of such experiments are due to the H^+ ion, the molecule or the anion of a particular acid—a distinction for which the necessity and basis were clearly indicated by Overton (1902). The free permeability of many cells to CO_2 has been shown by Jacobs (1920a, b), and Smith and Clowes (1924a) have emphasized the rôle of the unhydrated, dissolved gas, CO_2 in this penetration process.

The redistribution of ions, under physiological conditions, between cell and fluid is well established and known to play an important part in the physico-chemical equilibria of blood. The early experiments of Zuntz (1868) showed that the corpuscles are freely permeable to CO_2, and that when whole blood is treated with CO_2 the capacity of the serum to take up CO_2 is increased. Grüber (1895) showed that CO_2 leads to an increase in the alkali titratable with methyl orange in the serum; ash analyses indicated that no Na or K had passed into the serum, but that Cl had left it and entered the corpuscles, to be replaced by HCO_3. This shift of Cl and HCO_3 between corpuscles and serum has been confirmed by Koeppe (1897), Hamburger (1916), de Boer (1917), Van Slyke and Cullen (1917), McLean, Murray and Henderson (1920), Fridericia (1920), Doisy and Eaton (1921), Mukai (1921), Dautrebande and Davies (1923), Mellanby and Wood (1923) and others.

De Boer (1917) has shown that SO_4 can be made to pass from serum to cell when blood to which sulphate has been added is treated with CO_2. Whether or not there occurs a similar redistribution of cations under the same conditions is a question on which there is not agreement. Evidence has been advanced in the affirmative by Hamburger (1916), Mellanby and Wood (1923) and others.

By taking into account the exchange of Cl and HCO_3 between corpuscles and plasma, Van Slyke, Wu and McLean (1923) have been able, on the principles originally stated by Henderson (1908, 1909), to define the chemistry of the respiratory cycle of mammalian blood with a great degree of accuracy. Certain assumptions have been made by them which have their origin in the above facts and the intent of which has a particular bearing on the subject matter of this paper. These assumptions are to the effect that the corpuscle membranes are permeable to water, to carbon dioxide, to the inorganic anions, and to either H^+ or OH^- ions, or both; while they are impermeable to the proteins and to K^+ and Na^+ ions. While admitting that it is unsound to draw *a priori* a parallel between the properties of the highly differentiated blood corpuscle and the elementary and more general protoplasmic properties as manifested in egg cells, etc., the evidence for the permeability to the bicarbonate ion in both cases suggests that there may be a common physiological basis for the anion exchange between cell and fluid.

The program proposed above was carried out at Salisbury Cove during the summer of 1924. Neither *Asterias* nor *Arbacia* eggs were available on the Maine coast during the summer months, so that it was necessary to repeat the work on carbonic acid, and to do the other experiments reported here with the eggs of the sand dollar, *Echinarachnius parma*, which

can be obtained there in large quantities and which are quite as suitable for the purpose.

Methods. The method of conducting the experiments was essentially the same as previously described (Smith and Clowes, 1924a). Considerable care must be used in the selection of eggs, for unless the sand dollars are properly collected and kept in the laboratory under favorable conditions the eggs deteriorate rapidly, becoming polyspermic on fertilization, segmenting irregularly and possessing low viability. Such eggs show an abnormal sensitivity to inhibitory agents and are therefore worthless for quantitative work. It was found desirable to collect fresh sand dollars at least every other day and to bring them to the laboratory with as little crowding as possible in the collection buckets. Poor eggs have on a few occasions been traced to crowded buckets in which the water was densely stained by the characteristic green pigment. In the laboratory the sand dollars were well distributed in shallow troughs under running sea water.

Just prior to opening, the dollars were well washed with tap water. The edges of the test were cut away and the oral portion removed with tap-water-cleansed instruments. At the height of the season the eggs are shed abundantly, and can be obtained in pure suspensions by washing them from the ovaries with sea water. At other times shedding must be forced by removing the ovaries and cutting them into several fragments in sea water; the shed eggs can then be centered in the bowl by circular agitation and removed with a pipette. Eggs obtained in the latter manner are not so good as those which are freely shed, since they contain numbers of immature and unfertilizable eggs, but they can be used in necessity.

At all times the eggs from each sand dollar were collected and fertilized separately, using the smallest possible quantity of sperm. It was found advantageous to inseminate each lot two or three times, at intervals of three or four minutes, with a quantity of sperm hardly sufficient by itself to fertilize all the eggs. This insures to some extent against polyspermy. Ten or fifteen minutes after fertilization each lot was examined and the better lots selected for use. Those lots were rejected in which there were more than 2 or 3 per cent of unfertilized eggs (that is, eggs without fertilization membranes) and also lots in which there was an equal incidence of eggs in which the fertilization membrane adhered at one or more points to the cortex of the egg, or where the membrane was collapsed or crenated. Thin, easily collapsible membranes, and the tendency of membranes to adhere to the cortex were found to be fairly certain criteria of poor viability. When by such signs as these, and by the development of the controls, a lot of eggs is in prime physiological condition, the behavior of this lot with respect to such

experiments as are reported here is invariably consonant with the behavior of other, similar lots. This fact indicates that the deliberate selection of eggs does not lead to qualified, conditional results, but to results that have a uniform, constant physiological basis. The eggs were transferred shortly after fertilization to the solutions under examination and their subsequent development in these solutions was followed by removing and fixing samples at intervals and carefully counting the numbers of eggs in the various stages of development. A drop of 5 per cent formalin in 2 or 3 cc. of sea water was used as the fixative.

With few exceptions the experiments were performed with sea water from which all CO_2 had been removed. This "neutral sea water" was prepared by adding 2.4 cc. N HCl to each liter of sea water and aerating for 12 to 18 hours with a water vacuum pump. By this means all CO_2 is expelled except a trace in equilibrium with the air. This acidified sea water was then brought to pH 7.2 to 7.4 by the addition of NaOH. That neutral sea water prepared in this way is essentially free from all weak acids is shown by the fact that two drops of 0.1 N HCl when added to 100 cc. bring it to pH 4.5 to 5.0.

To this neutral sea water sodium bicarbonate, sodium acetate, etc., were added from stock solutions in the desired quantities. The resulting solutions were then divided into 100 cc. portions and the hydrogen-ion concentration adjusted by the addition of HCl of the appropiate strength. When carbonic acid was present the experiments were carried out in glass stoppered bottles holding 101 to 103 cc. A quantity of the solution was placed in the bottle with the eggs, the desired amount of HCl added and the mixture then made up to 100 cc. In the absence of carbonic acid the HCl was added to 100 cc. of the solution contained in open bowls.

The stock solution of sodium bicarbonate was prepared from the c.p. salt; it was 0.975 normal in respect to total CO_2, as determined by analysis with a Van Slyke apparatus, and 0.985 normal in respect to total base, as determined by hot titration with bromcresol purple. The sodium acetate solution was prepared from the salt, and proved by electrometric analysis to be 1.0 $\pm$ 0.01 normal, using the standard acetate data given by Michaelis (1922). The sodium propionate solution was prepared from the technical salt and was judged from electrometric analysis to be 1.0 $\pm$ 0.05 normal. The sodium lactate solution was prepared by the neutralization of lactic acid, and the other salt solutions from their respective salts.

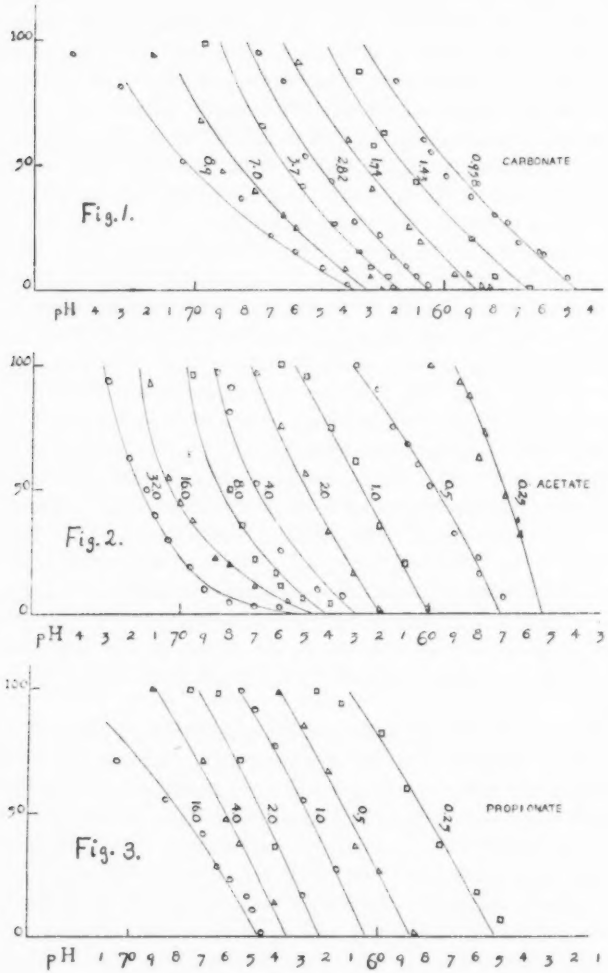
The hydrogen ion concentration of all mixtures was determined colorimetrically during the course of the experiments with a set of prism standards which were subsequently standardized against sea water solutions, the reaction of which was determined with a hydrogen electrode. The salt errors in the particular standards used were found to be +0.1 for

bromthymol blue and bromcresol purple and +0.35 for cresol red, bromcresol green and bromphenol blue. Kolthoff (1922) states that the salt error of bromcresol purple in 0.5 N NaCl is +0.25; the smaller salt effect observed here may be attributable to fading of the indicator in the standard. A further check of the colorimetric values was obtained on the critical carbonate solutions given in table 2 by determining the hydrogen ion concentration with the quinhydrone electrode (Biilmann, 1921). With the exception of the hydrogen ion, all concentrations are expressed in this paper in *millimols per liter* (mM.p.l.).

Carbonic, acetic and propionic acids. Experiments with these acids are illustrated in figures 1, 2 and 3. Each contour line represents a set of simultaneous experiments in which the fertilized eggs were immersed in sea water solutions containing a definite quantity of the sodium salt of the acid under investigation (indicated by the adjacent figure) and sufficient HCl to bring them to the hydrogen ion concentration shown. The degree of development (ordinates in the figures) relative to a simultaneous sea water control was determined by counting "fixed" samples of the eggs removed from these solutions when the controls had reached the 8 or 16 cell stage. (In the case of acetic and propionic acids the effective concentrations of acid exist at reactions at which these acids have little or no buffering capacity. Since a considerable degree of stability of reaction is demanded, a small quantity of phosphate (0.4 mM.p.l.) was added. The quantity of phosphate was made small with deliberate caution, but as will be shown presently, the presence in the fluid of the ions $H_2PO^-_4$ and $HPO_4^=$ does not effect the intracellular equilibria.)

The effect of increasing hydrogen-ion concentration in each of these solutions is to decrease progressively the rate of cell division until finally this process is completely arrested. Consequently samples of eggs taken from these solutions at intervals after their immersion, show varying degrees of development according to the extent to which the division process has been retarded. (The time required for the eggs to come to the new and constant rate of division after being placed in the acidified solutions is very short; as nearly as can be measured, not more than two to three minutes. Since in the experiments given here the eggs were left in the solutions for one and a half to several hours, the time necessary to attain equilibrium between the cells and fluid cannot influence the results, and has only theoretical interest.)

It has already been shown for *Arbacia* and *Asterias* (Smith and Clowes, 1924b), and will presently be shown for *Echinarachnius* eggs, that this inhibition of cell division cannot be attributed to the increased concentration of hydrogen ions, *per se*, in the fluid, because these eggs will divide with normal velocity at much greater acidities (pH 5.3) than are involved



Figs. 1, 2 and 3. Each contour line represents a set of simultaneous experiments in which the fertilized eggs were allowed to develop in sea water solutions initially containing the same concentration of NaA (as shown by the adjacent figure) and to which sufficient HCl had been added to bring the solution to the pH indicated on the abscissae. The ordinates indicate the degree of development, relative to a simultaneous sea water control, determined by accurate counts made when the controls had reached the 8 or 32 cell stage.

here when only HCl is present in the solution. Instead the inhibition must be attributed to the presence in the solutions of free carbonic acid, acetic acid, or propionic acid, as the case may be, and to the power of these acids to enter the cell under conditions where HCl cannot enter. Consequently the increasing inhibition associated with increasing hydrogen-ion concentration illustrated in the above figures expresses simply the effects of increasing concentration of weak acid liberated by the interaction between the salt of the weak acid present and the added HCl. In this view, it might be expected that the same concentration of weak acid would have the same physiological effect regardless of the presence of other ions. If such were the case, the solutions having the same physiological effect should differ by +0.3 pH every time the total weak acid-salt concentration is doubled, because such solutions have the same content of free weak acid. That such is not the case is perfectly clear from the above figures; the effectiveness of the weak acid depends on the presence and concentration of the salt, and, therefore, indirectly on the hydrogen-ion concentration. In order to analyze the relations existing between these variables we must consider the composition of these solutions in terms of the concentrations of the weak acid and its anion which are in equilibrium at the observed hydrogen-ion concentration. For the present we will consider the composition of only those solutions which just completely repress cell division, and not attempt any quantitative analysis of solutions which partially but incompletely repress it. The hydrogen-ion concentration at which cell division is just completely repressed in any solution containing a given total concentration of carbonate, etc., is obtained by extrapolating to zero development the contour line which connects the points denoting the degree of development at various hydrogen-ion concentrations (cf. above figures). This is a more accurate method of obtaining this critical value than direct observation because of the fragmentation and abortive division which almost invariably occur in some eggs at this time.

From this hydrogen ion concentration, $[H^+]$, and from the dissociation constant of the weak acid involved, k, the degree of dissociation of the weak acid salt, γ, and the total concentration of the weak acid in the solution $[BA]_0$, all of which are known, we can calculate the concentrations of free acid [HA] and its anion $[A^-]$ by rearranging the mass law equation 1 and the summation 2 into the forms 3 and 4:

(1) $$[H^+] = \frac{[HA]}{[A^-]}\, k$$

(2) $$[BA]_o = [HA] + \frac{[A^-]}{\gamma}$$

(3) $$[HA] = \frac{[BA]_o}{1 + \frac{k}{[H^+]\,\gamma}}$$

$$[A^-] = \frac{\gamma\,[BA]_o}{1 + \frac{[H^+]\,\gamma}{k}} \tag{4}$$

In the calculations performed in this paper the following constants were used:

TABLE 1

	k
Carbonic acid (first)	3.25×10^{-7}
Acetic acid	1.82×10^{-5}
Propionic acid	1.4×10^{-5}
Benzoic acid	6.6×10^{-5}
Salicylic acid	1.0×10^{-3}
Ionic activity of sea water	0.72

γ for uni-univalent salts in sea water 0.618.

k for carbonic acid was obtained by interpolation to 20° of Kendall's data (1916); k for the other acids are the commonly accepted values.

TABLE 2

Carbonic acid

$[CO_2]_o$	$[BA]_o$	HCl*	[pH]	$[HA]_T$	$[A^-]$	(pH)
0.958	0.969	1.7	5.45	0.834	0.076	6.57
1.425	1.44	2.25	5.65	1.155	0.168	6.45
1.745	2.14	3.0	5.86	1.268	0.298	6.47
2.82	2.86	3.5	6.07	1.743	0.665	6.45
3.74	3.77	4.2	6.17	2.1	1.01	6.45
5.37	5.78	5.8	6.25	2.78	1.60	6.44
7.06	7.52	7.25	6.31	3.42	2.26	6.48
8.9	9.24	8.4	6.36	4.04	3.01	6.48
			Asterias and *Arbacia* (Smith and Clowes)			
1.75	2.13	3.0	(5.96)	1.22‡	0.33	6.49
3.44	4.01	4.45	(6.27)	1.82	1.0	6.51
5.09	5.85	5.8	(6.37)	2.44	1.64	6.51
6.68	7.60	7.25	(6.41)	3.04	2.24	6.50

* Cubic centimeters N/20 HCl in 100 cc. mixture required to completely repress cell division.

† [HA] and $[A^-]$ calculated from obs. $[H^+]$ by equations 3 and 4.

‡ [HA] and $[A^-]$ calculated from obs. CO_2 tension.

The ionic activity of sea water was calculated by the square of the valence rule (Lewis and Randall, 1923) and γ obtained for this value by interpolation of Noyes and MacInnes' data on KCl (1916). Electrometric titrations of these acids in sea water have closely confirmed this value of γ.

The data relative to carbonic, acetic and propionic acids are given in tables 2 and 3, and figure 4. With these data are included some previously recorded by Smith and Clowes (1924a) for the action of carbonic acid on *Asterias* and *Arbacia* eggs.

From these data it will be observed, first, that as the concentration of anion in the solutions increases, increasing concentrations of acid are required to inhibit cell division. Therefore the inhibitory effect cannot

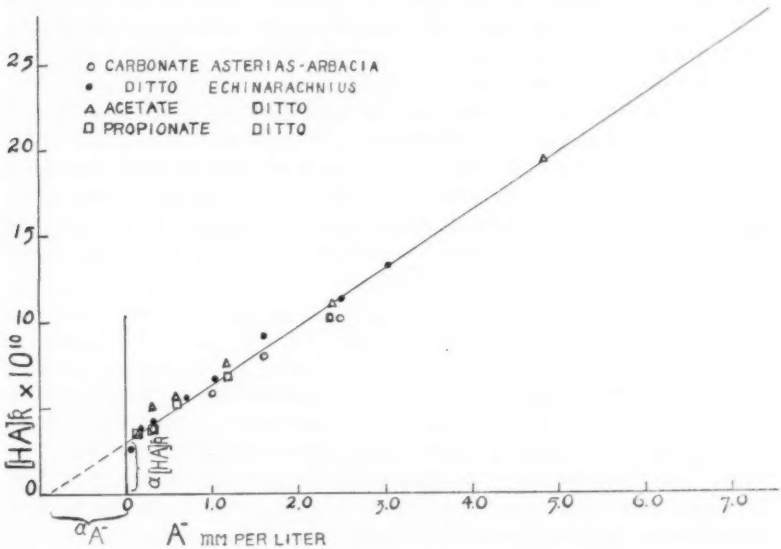


Fig. 4. The critical concentrations of carbonic, acetic and propionic acids required to just completely repress cell division, multiplied by the respective dissociation constants (ordinates) plotted against the equilibrium concentrations of anion (abscissae). Notice that [HA]k increases in simple proportion to [A$^-$], that $\frac{[HA_1]k}{[A^-]} = \frac{[HA_2]k_2}{[A_2]}$, and that when the concentration of anion is zero, a considerable concentration of acid is still required to repress cell division (indicated by $\alpha_{[HA]k}$). The significance of these facts is discussed in the text.

be attributed to the free acid alone. Secondly, it will be observed that though the hydrogen-ion concentration decreases rapidly as the concentration of anion increases in the inhibitory solution, it does not decrease uniformly but at a rapidly diminishing rate and in a manner which shows clearly that it is approaching a limiting asymptote (about pH 6.5). On the other hand the rate of increase in the concentration of anion is uniformly related and in simple proportion to the rate of increase in the concentration of free acid. This is shown more clearly by the arrange-

ment in figure 4, where the critical concentrations of free acid, multiplied by the respective dissociation constant, are plotted against the corresponding or equilibrium concentrations of anion. (The values of [HA] k rather than [HA] have been used so that these values can be plotted in one graph and to the same scale for comparison.)

It is shown by the above data that at zero or any finite anion concentration, the concentration of acetic acid required to repress cell division and the concentration of carbonic acid similarly required at the same anion concentration are inversely related as the respective dissociation constants of these acids; and that a similar inverse proportion holds between propionic and carbonic acid, or between propionic acid and acetic acid. It follows from this fact that the inhibition of cell division caused by these acids is strictly a hydrogen-ion effect depending on their properties as acids, and not a consequence of any specific physical or molecular action. For, from the mass law, the concentrations of two acids required to produce the same hydrogen-ion concentration in the presence of equal concentrations of their respective anions are inversely related as their dissociation constants. That is

$$[H^+] = \frac{[HA_1]k_1}{[A_1^-]} = \frac{[HA_2]k_2}{[A_2^-]} = \frac{[HA_3]k_3}{[A_3^-]} \tag{5}$$

That the "site" of action of these acids is not on the "exterior" of the cell is clear from the fact that HCl and other acids (as will be seen presently) have no inhibitory action at the same hydrogen-ion concentrations. Therefore we say that these acids "penetrate" the cell since they exert a hydrogen-ion effect at some point in the cell which is not equally accessible to other acids, or to hydrogen ions *per se*.

Since in each case the concentration of the acid required to repress cell division increases as the concentration of anion in the fluid increases, it is inferred that the anions must also participate in this "intracellular" action, and therefore must "penetrate" the cell. (It will be shown subsequently that we are probably not dealing with the actual migration of anion from fluid to cell, but that the concentration of anion in the cell is established by some other means; but so far as the equilibrium concentrations are concerned, the appearance of the anion in the cell may be treated as a consequence of actual migration.)

From these facts we can consider what hydrogen-ion concentration the various mixtures given in tables 2 and 3 set up in the cell. To determine this we must know the equilibrium relations between the concentrations of acid and anion in the cell and in the fluid. No direct evidence on these relations is available, but from a few general propositions they can be predicted with good approximation.

Entirely different laws determine the equilibrium distribution be-

tween cell and fluid of the molecules of free acid on the one hand and the anions on the other. It seems probable that they gain access to the cell through separate channels, the penetration of the acid molecules (or of anhydrous CO_2) depending on diffusion or an allied physical process, and the penetration of the anions depending on transportation by chemical combination. With regard to the acid molecules, it is a general view that their penetrating power, like that of other molecular substances, is determined in part at least by their physical properties; certain properties singly or together being necessary for penetration. This circumstance arises, according to this view, from the particular nature of the channels through which penetration is effected. Accordingly we should expect the particular character of any substance, whether favorable or unfavorable to penetration, and the particular nature of the channel of penetration, whether aqueous or non-aqueous, to influence the rate at which equilibrium is attained, since these factors will determine the actual quantity passing from fluid to cell or cell to fluid in unit time. But, from a consideration of Henry's law, we would not expect these factors to influence the final distribution of the substance between cell and fluid; the cause of penetration is an initial difference in effective concentration in cell and fluid, and at equilibrium these effective concentrations must be equalized regardless of what physical forces, processes or menstrua are involved in the act of penetration. (The effective concentration of a non-electrolyte with respect to diffusion pressure may be roughly considered as proportional to the product of the real concentration times the reciprocal of the solubility.) If the properties of the menstruum in the cell are such that the effective and real concentrations are so related as they are in the fluid, then at equilibrium, the real concentrations in the cell and fluid must also be equal. From the recognized condition of approximate osmotic equality between cell and fluid, we may infer that this last condition is true, and no serious error will be introduced by stating the concentration of free acid in the cell (under equilibrium conditions) equal to the concentration in the fluid. Using *round brackets* to denote intracellular concentrations and *square brackets* to denote concentrations in the fluid, we therefore write for the acid molecules, [HA],

$$(HA) = [HA] \tag{6}$$

(It is assumed here and in all other similar equations in this paper that the actual quantities of reactant in the fluid is not increased or decreased by the passage of reactant from fluid to cell or cell to fluid. This, of course, is experimentally established by the circumstance that a very small volume of cells is added to a large volume of solution. Equation 6 may not hold when [HA] is small and when CO_2 is being produced

by metabolism in the cell. But when [HA] is large and all perceptible cellular activity is repressed, an appreciable inequality from this source is unlikely.)

Unlike the distribution of molecules, the distribution of anions between cell and fluid cannot be taken as equal, for the effective concentrations of the diffusible ions in the cell (or fluid) will be influenced by the presence of non-diffusible ions, such as proteins and non-diffusible acids, in the direction and degree defined by Donnan's law. (For a discussion of the Donnan effect see Lewis, 1920.) This unequal distribution results from the fact that the effective concentration of any ion is dependent on the presence of oppositely charged ions, and consequently if there is an unequal distribution of one species of ion, all other species will be thereby affected. It may be noted that this unequal distribution of diffusible ions is determined without regard to the nature of the membrane or to the *modus operandi* of penetration. Conversely there is no reason to believe that the applicability of the Donnan law would be modified by the particular nature of the means by which an ion gains access to the cell.

With regard to a possible unequal distribution of anions between cell and fluid in the case under consideration, nothing more than the theoretical conditions can be stated. It is not known whether the proteins of the cell exist, under the conditions of these experiments, as anions or cations. In addition to the proteins, but probably to a lesser degree, any non-diffusible inorganic cations and anions which may be present would contribute to the unequal distribution. But whatever the cell-fluid ratio for diffusible anions may be, it will apply equally to all diffusible anions, and indicating this Donnan ratio by r, we may write:

$$(7) \qquad r = \frac{(HCO_3^-)}{[HCO_3^-]} = \frac{(C_2H_3O_2^-)}{[C_2H_3O_2^-]} = \frac{(C_3H_5O_2^-)}{[C_3H_5O_2^-]} = \frac{(A^-)}{[A^-]}$$

So far as the proteins are concerned the actual value of this ratio will depend on the quantity of cation or anion combined with protein, which in turn will depend on the equivalent concentration of protein in the cell, on its combining properties and on the hydrogen-ion concentration. None of these factors is known, but the first two presumably will be constant in various experiments with the same egg cells and (anticipating the demonstration of this fact) the intracellular hydrogen-ion concentration remaining constant, the Donnan ratio will have a constant value in all experiments considered here. (This is conditional, of course, on a constant concentration of salt in the external fluid, which is approximately realized in these experiments.) We may therefore write for the intracellular anion concentration:

$$(8) \qquad (A^-) = r\,[A^-]$$

Since the Donnan ratio enters uniformly into all the equations, we will, in the absence of definite information about its true value, ascribe to it a value of unity for tentative calculations. Should the value be as low as 0.7 or as high as 1.3 (which seem probable lower and upper limits) the resulting calculations of intracellular hydrogen-ion concentration will not be altered by more than ± 0.15 pH.

The data on carbonic, acetic and propionic acids confirm the applicability of equation 8, in as much as they clearly show that the intracellular concentration of anion increases in constant proportion to the extracellular concentration. But that this equation does not entirely cover the situation is shown by the fact that when the concentration of anion in the fluid is zero a considerable quantity of acid (indicated in fig. 4 by $\alpha_{[HA]k}$) is still required to repress cell division. This concentration of acid is much greater than that which would be required in a simple acid solution to establish the hydrogen ion concentration obtaining in the cell under these conditions, even when the latter is estimated with the greatest latitude for error. There is no fact, either in these data or in general experience, which explains this extrapolation value; the only conclusion that can be drawn from the data is that this value has its origin in a chemical rather than a physical process, since the inhibitory concentrations of the three acids at zero anion concentration are related inversely as their dissociation constants. That is

$$\alpha_{H_2CO_3} k_1 = \alpha_{HAc} k_4 = \alpha_{HPr} k_3 \tag{9}$$

Or more simply, if expressed in terms of $[A^-]$ instead of [HA] (cf. fig. 4),

$$\alpha_{HCO_3} = \alpha_{Ac^-} = \alpha_{Pr^-} \tag{10}$$

The definite and constant form of this extrapolation value indicates, broadly, a capacity on the part of the cell to resist the acidifying action of the acid in spite of an apparent condition of free permeability to both acid and anion. This departure, whatever its cause, appears in the stoichiometrical equation as a constant quantity of anion, α_{A-}, in the cell in excess of the concentration of anion in the fluid. Therefore it appears that so far as the stoichiometrical equation is concerned we may consider this resistance as a virtual quantity of (A^-), and by introducing the appropriate value in the mass law equation calculate the intracellular hydrogen-ion concentration. We therefore write for this value, combining equations 6 and 8, and introducing the term $\alpha_A{}^-$,

$$(H) = \frac{[HA]}{r(\alpha_{A^-} + [A^-])} k \tag{11}$$

(The Donnan ratio, r, is equally applicable to $_A{}^-$ and $[A^-]$, since $\alpha_A{}^-$ is expressed in terms of $[A^-]$.)

From the extrapolation of the data in tables 2 and 3, α_{A^-} is given a value of 0.9 mM.p.l. The values of the intracellular hydrogen-ion concentration, (pH), given in tables 2 and 3, were calculated by using this value, and by making the Donnan ratio, r, equal to 1.0. It is noteworthy that the effect of α_{A^-} in equation 11 is such as to make the cell always more alkaline than the fluid, the difference between (pH) and [pH] becoming larger as $[A^-]$ decreases. This is just the reverse of what would happen if the anion distribution were governed primarily by the Donnan law, for in this case the cell would always be more acid than the fluid, and (pH) and [pH] would differ by a constant amount, $\log \frac{1}{r}$, which probably would not exceed 0.3 pH.

TABLE 3

a. Acetic acid

$[BAc]_0$	[pH]	[HAc]	$[Ac^-]$	(pH)
0.224	5.54	0.02	0.126	6.45
0.470	5.72	0.0286	0.273	6.35
0.966	6.0	0.0318	0.578	6.41
1.96	6.2	0.0413	1.18	6.44
3.94	6.3	0.0662	2.40	6.44
7.90	6.4	0.106	4.82	6.47
15.8	6.47	0.180	9.65	6.51
31.6	6.50	0.336	19.30	6.52

b. Propionic acid

$[BPr]_0$	[pH]	[HPr]	$[Pr^-]$	(pH)
0.222	5.53	0.0257	0.12	6.45
0.472	5.87	0.0267	0.27	6.50
0.963	6.05	0.0368	0.57	6.46
1.95	6.23	0.0496	1.18	6.48
3.93	6.37	0.073	2.39	6.51
14.79	6.47	0.22	9.0	6.51

It seemed that it might be possible to throw further light on the significance of α_{A^-} by observing the simultaneous effects of two of the above acids on cell division. Consequently a number of experiments were performed with mixtures of carbonic and propionic acids. These experiments with mixtures have the further value of affording a control against the phosphate used in buffering the acetate and propionate solutions of tables 2 and 3, since the carbonate serves as an effective buffer.

These experiments with mixtures are given in figures 5 and 6, and summarized in table 4. Reference to these data shows that as the concentration of acetate increases, cell division is inhibited at diminishing concentrations of carbonic acid and increasing concentrations of carbonate

ion. Since a constant intracellular hydrogen ion concentration could not be established by these carbonic acid and carbonate ion concentrations in conjunction with any constant value of $\alpha_{HCO_3^-}$ it is apparent that $\alpha_{HCO_3^-}$ does not have a constant value under these circumstances, but a variable value depending on the relative quantities of carbonic and acetic acid in the system. In accordance with the suggestion that α_A^- could be treated as a definite quantity of anion present in the cell in excess of that present in the fluid, we suppose that the base, α_{B^+}, combined with this quantity of anion, and which was combined solely with one anion when only one acid was present, is now in the presence of two acids distributed between the two anions.

The method of distribution of this base to be expected from the mass law can be obtained as follows; we write the summation:

$$r\alpha_{B^+} = r\alpha_{A_1^-} + r\alpha_{A_2^-} \tag{12}$$

Since in the cell (HA_1), $\alpha_{A_1}^-$, (HA_2) and $\alpha_{A_2}^-$ must be in equilibrium with the same hydrogen ion concentration, equation 11 can be written, when $[A^-]$ = zero,

$$(H) = \frac{(HA_1)k_1}{r\alpha_{A_1}} = \frac{(HA_2)k_2}{r\alpha_{A_2}} \tag{13}$$

Then

$$r\alpha_{A_1^-} = r\alpha_{B^+} - \frac{(HA_2)k_2}{(H)} \tag{14}$$

$$r\alpha_{A_1^-} = r\alpha_{B^+} - \frac{(HA_2)k_2}{(HA_1)k_1} r\alpha_{A_1} \tag{15}$$

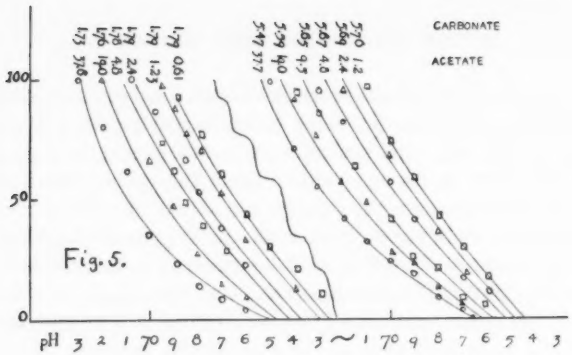
$$r\alpha_{A_1^-} = \frac{r\alpha_{B^+}}{1 + \frac{(HA_2)k_2}{(HA_1)k_1}} \tag{16}$$

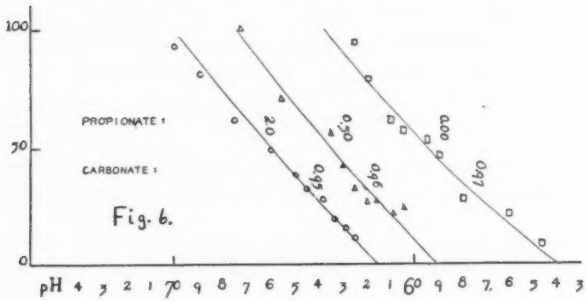
Applying equation 6 to both (HA_1) and (HA_2), and equation 8 to both $[A_1^-]$ and $[A_2^-]$, the intracellular hydrogen ion concentration becomes

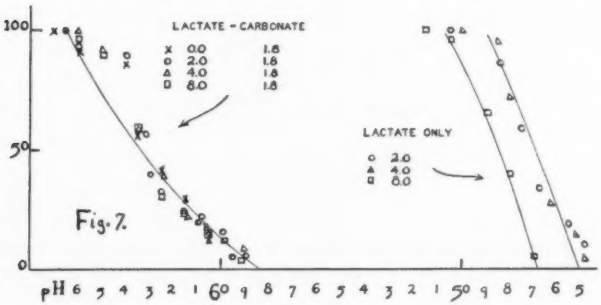
$$(H) = \frac{[HA_1]}{\frac{r\alpha_{B^+}}{1 + \frac{[HA_2]k_2}{[HA_1]k_1}} + r[A_1]} k_1 \tag{17}$$

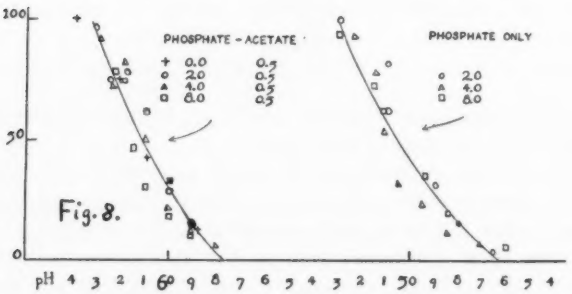
(H) can be expressed in terms of $[HA_2]$ by performing the alternate substitutions in equations 14 and 15.

The values for the intracellular hydrogen ion concentration given in table 4 were calculated by making $r = 1.0$, as before, and $\alpha_B^+ = 0.9$ mM.p.l., as found for each acid separately. The constancy of (pH) obtained from these mixtures confirms the stoichiometrical treatment accorded this extrapolation constant, α, and shows that the egg cell is









Figs. 5, 6, 7 and 8. See figure 1 and text.

"permeable" to both the free acid and the anion of carbonic, acetic and propionic acids, whether these acids are present singly or in mixtures.

Lactic, phosphoric and tartaric acids. The circumstance that the egg is apparently freely permeable to the anions of carbonic, acetic and propionic acids as well as to the free acids raises the question whether this apparent permeability to anions is a general rule, or only applicable to certain species. As appropriate substances for examination of this question lactic acid was chosen because of its small penetrating power; and tartaric and phosphoric acids because, besides having small pene-

TABLE 4

a. Carbonate-acetate mixtures

$[BHCO_3]_0$	$[BAc]_0$	[pH]	$[H_2CO_3]$	$[HCO_3^-]$	[HAc]	$[Ac^-]$	$\frac{[H_2CO_3]\,k}{[HAc]\,k}$	(pH)
1.79	0.613	6.25	0.92	0.539	0.0113	0.372	1.45	6.55
1.79	1.23	6.28	0.895	0.555	0.0215	0.748	0.745	6.51
1.79	2.42	6.37	0.802	0.611	0.0346	1.47	0.415	6.53
1.79	4.85	6.42	0.753	0.641	0.0619	2.96	0.218	6.52
1.78	9.6	6.44	0.727	0.651	0.1168	5.85	0.111	6.50
1.76	19.0	6.47	0.69	0.661	0.218	11.6	0.0565	6.50
1.73	37.6	6.49	0.639	0.674	0.409	22.9	0.0279	6.50
5.70	1.23	6.43	2.36	2.06	0.0154	0.748	2.74	6.55
5.69	2.41	6.48	2.20	2.15	0.0272	1.47	1.45	6.57
5.67	4.85	6.52	2.06	2.22	0.0493	2.97	0.748	6.59
5.65	9.55	6.56	1.94	2.29	0.0885	5.89	0.392	6.61
5.59	19.0	6.61	1.78	2.36	0.1585	11.7	0.200	6.63
5.47	37.7	6.65	1.63	2.38	0.2860	23.2	0.102	6.66

b. Carbonate-propionate mixtures

$[BHCO_3]_0$	$[BPr]_0$	[pH]	$[H_2CO_3]$	$[HCO_3^-]$	[HPr]	$[Pr^-]$	$\frac{[H_2CO_3]\,k}{[HPr]\,k}$	(pH)
0.97	0.00	5.40	0.855	0.069				6.54
0.96	0.49	5.90	0.679	0.174	0.0176	0.301	0.895	6.50
0.95	1.96	6.15	0.546	0.249	0.0468	1.205	0.269	6.46

trating power, they present the novel condition that the acid component, with respect to the divalent secondary ions, is an ion rather than a molecule. Thus the demonstration of an intracellular acid effect in the system $H_2PO_4^-$:$HPO_4^=$ would be substantial evidence of the actual migration of the $H_2PO_4^-$ ion from the fluid into the cell.

The results of experiments with these acids, which were performed in the same manner as those previously described, are given in figures 7, 8 and 9.

These experiments lead to the conclusions that neither the acid com-

ponents ($HC_5H_5O_3$, $H_2PO_4^-$ or $HC_4H_4O_6^-$) nor the opposed anions ($C_3H_5O_3^-$, $HPO_4^=$ or $C_4H_4O_6^=$) penetrate the egg cell to any appreciable extent, and that in solutions containing them cell division is impaired only when the hydrogen ion concentration of the fluid reaches a limiting value incompatible with cell division.

These conclusions are based on experiments in which cell division was followed in sea water solutions containing these acids singly, and also on experiments in which mixtures were used consisting of one of these acids with either carbonic or acetic acid. Experiments of both types are illustrated in the above figures.

As shown by these data, when lactate, phosphate or tartrate is present in the solution, cell division occurs with normal velocity until the hydrogen ion concentration of the solution reaches pH 5.2 to 5.0. Cell division is impaired by further increases in acidity, and completely repressed at pH 4.6 to 4.8. A two- or three-fold increase in the total concentration of weak acid has no significant influence on the value of this limiting acidity. This fact is considered to be ample demonstration and corroboration of the general experience that the penetrating power of these acids is of a very low order. To insure against the possibility that these acids might penetrate slowly and that our experiments did not allow sufficient time for penetration, the eggs were transferred, in the experiments shown, to the solutions as soon after fertilization as possible, and their development was observed quantitatively up to the 64-cell stage and qualitative observations made 24 hours later. In all cases at pH 5.6 to 5.8 the eggs developed to swimmers, which, though greatly behind the controls, had advanced and were well formed.

The fact that the limiting acidity is approximately the same with each acid makes it extremely probable that this acidity (pH 4.6) represents the limiting concentration of hydrogen ions, *per se*, which is compatible with cell division. This point was tested by the addition of dilute HCl to neutral sea water containing no weak acid. In every case cell division occurred normally to the 8 cell stage at pH 5.2 and was completely repressed at pH 4.6. No cytological examination of these eggs was made, but it can be said that there is no gross difference in the appearance of eggs inhibited by HCl, $H_2PO_4^-$, etc., at pH 4.6 and those inhibited by carbonic acid at pH 6.0 to 5.6, except that in the former case there is a greater incidence of segmented eggs containing a divided nucleus. As nearly as can be judged, the rate at which inhibition is effected by HCl at pH 4.6 is of the same order of magnitude as in the case of carbonic acid, complete arrest of cell division occurring within a few minutes. The inhibition effected by HCl is also, to some extent at least, reversible, though whether it is as reversible as that of carbonic acid, cannot be said.

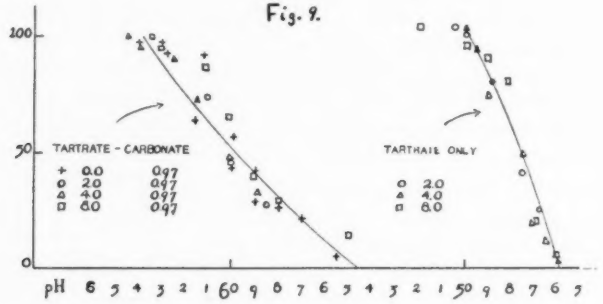
Regarding the action of HCl, it might be supposed that above a certain concentration of hydrogen ions the semipermeable properties of the cell are so modified that the hydrogen ion migrates from fluid to cell; or it might be supposed that HCl inhibition is an indirect consequence of some action on the egg cortex. Lastly, the possibility remains that there is at all hydrogen-ion concentrations, an equilibrium between cell and fluid with respect to the hydrogen ion which involves a concentration gradient of such an order that an external hydrogen-ion concentration of pH 4.6 is required to establish the critical acidity within the cell.

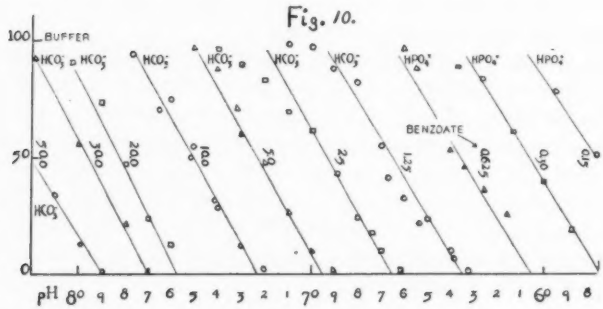
In any case, the extreme acidity required in these solutions to repress cell division is a conclusive demonstration that the distribution of these particular ions (H^+, $H_2PO_4^-$ and $HC_4H_4O_6^-$) between cell and fluid is not primarily in accordance with the Donnan law. The inapplicability of this law to the distribution of the "diffusible" anions (HCO_3^-, Ac^- and Pr^-) has already been pointed out. It remains to be determined whether the Donnan equation holds under the special terms of an equilibrium such as that defined by equation 17.

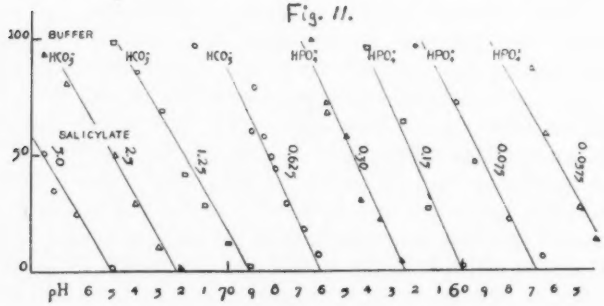
Lactate anion, and the secondary phosphate and tartrate anions. The above evidence is sufficient to exclude the penetration of lactic acid, and of $H_2PO_4^-$ and $HC_4H_4O_6^-$ at hydrogen-ion concentrations less than pH 4.6, but offers no information on the possible penetration of the lactate anion, or $HPO_4^=$ or $C_4H_4O_6^=$ ions. If these ions penetrated without the opposing acid they would cause no increase, and might, in the case of $HPO_4^=$, cause a decrease, in intracellular acidity. So the normal development of eggs in acid or neutral solutions containing these ions affords no information on their penetration.

This question can be directly tested, however, by mixtures containing these ions and an acid which is known to penetrate. Let us suppose, for example, that the eggs are immersed in a solution containing both carbonate and lactate, and that the lactate ion penetrates as well as the carbonate ion and carbonic acid; then the lactate ion will react in the cell with the carbonic acid to form lactic acid; more lactate ion and more carbonic acid will pass from fluid to cell, and the process will continue until an equilibrium will be reached in which the concentrations of carbonic acid, carbonate ion and lactate ion in the cell are in equilibrium with the corresponding concentrations in the fluid, according to equations 6 and 8; and in which both the lactate and the acetate in the cell will be in equilibrium with the same hydrogen-ion concentration, in accordance with equation 5. This is precisely the same condition as would result if lactic acid as well as the lactate ion entered the cell by direct migration from the fluid. Consequently if the lactate ion penetrates, the addition of sodium lactate to carbonate solutions should displace the carbonate equilibria in the manner observed with sodium acetate, and to the degree defined by equation 17.

When sodium lactate, sodium phosphate and sodium tartrate are added to carbonate mixtures, or to acetate mixtures, the equilibrium conditions of the latter are not perceptibly displaced (see figs. 7, 8 and 9); and con-







Figs. 9, 10, and 11. See figure 1 and text.

sequently it is concluded that the $C_3H_5O_3^-$, $HPO_4^=$ and $C_4H_4O_6^=$ ions do not penetrate to any greater extent than do lactic acid and the $H_2PO_4^-$ and $HC_4H_4O_6^-$ ions.

Benzoic and salicylic acids. The acids which have so far been considered fall into two classes. In the first class it appears that both the free acid and the anion penetrate the cell freely, and the effects of these acids on cell division are attributable solely to their participation in the intracellular acid-base equilibria. In the second class, neither the acid component nor the opposed anion penetrate the cell, and these appear to be without effect on cell division at moderate concentrations except as they participate in the acid-base equilibria of the surrounding fluid.

TABLE 5

Benzoic and salicylic acids

a. Benzoic acid

$[BA]_0$	[pH]	$[A^-]$	[HA]
0.15	5.4	0.089	0.0059
0.30	5.75	0.183	0.0056
0.625	6.05	0.383	0.0051
1.25	6.35	0.770	0.0055
2.5	6.65	1.55	0.0053
5.0	6.95	3.09	0.0053
10.0	7.23	6.18	0.0052
20.0	7.57	12.4	0.0052
30.0	7.7	18.5	0.0049
50.0	7.9	30.9	0.0054
Mean			0.0053

b. Salicylic acid

0.0375	5.34	0.023	0.000106
0.075	5.68	0.046	0.000097
0.15	6.0	0.093	0.000093
0.30	6.23	0.185	0.000106
0.625	6.62	0.386	0.000093
1.25	6.92	0.772	0.000093
2.5	7.22	1.55	0.000093
5.0	7.5	3.09	0.000098
Mean			0.000097

Benzoic and salicylic acids demonstrate the existence of a third class, in which the undissociated acid molecule possesses "specific" toxic properties which are so great that both of the above effects are completely obscured. In solutions containing sodium benzoate or sodium salicylate, cell division is repressed at constant concentrations of the acid molecule, HA, independently of all ion species in the system.

Data illustrating experiments with benzoic and salicylic acid are given in figures 10 and 11 and summarized in table 5. These experiments were

performed by adding various quantities of sodium benzoate or sodium salicylate to neutral sea water containing either $NaHCO_3$ or NaH_2PO_4 for buffer, and adjusting the reaction with HCl or NaOH.

It is apparent from the mass law that the concentration of an acid is determined by the total concentration of the salt of that acid and the concentration of hydrogen ions. If the concentration of salt is doubled, the hydrogen-ion concentration required to furnish a fixed concentration of free acid is reduced by one-half or the pH increased by 0.3. Thus in the experiments shown, the total benzoate or salicylate is in most instances doubled in successive solutions, and the pH in these solutions which is required to just repress cell division is correspondingly 0.3 more alkaline.

The slope of the curve relating development to the pH of the solutions is comparatively steep; a decrease of 0.5 pH near the critical concentration of acid converts a wholly innocuous solution to one in which cell division will be instantly arrested. Consequently the accurate control of the reaction of these solutions is necessary to the demonstration of these relations. Since at the reactions considered here these acids have no buffering capacity, carbonate and phosphate were used for this purpose. Carbonate (natural sea water) was used above pH 6.5, because in solutions more alkaline than pH 7.0 phosphate precipitates the calcium from sea water. At these reactions the inhibitory effect of the carbonate is negligible. Below pH 6.5 phosphate was used because in these more acid solutions a sufficient concentration of carbonic acid would exist to inhibit cell division.

Apart from the quantitative relations disclosed by these experiments, it would be difficult to say whether the action of these acids in repressing cell division was analogous to that of carbonic and acetic acid, or of a wholly different nature. The appearance of eggs which are partly or just completely inhibited by benzoic or salicylic acid is not sensibly different from that of eggs inhibited by carbonic acid. With concentrations of benzoic and salicylic acid greater than those required to repress division, the egg cell undergoes a series of cytolytic changes which differ somewhat according to the concentration of acid, varying from incipient cortical cytolysis, through granulation and liquefaction, to a condition resembling acid fixation.

With lesser concentrations of acid, such as are just required to repress the division process, the inhibitory action of benzoic acid and salicylic acids appears to be nearly as reversible as that of carbonic acid. The majority of eggs, in lots which were placed in benzoic acid solutions before the first cleavage plane and left for one hour, resumed development on return to sea water and formed fairly good swimmers. Fifty per cent, or better, of eggs exposed two hours recovered similarly.

The magnitude of the inhibitory concentrations involved with benzoic and salicylic acid, and the fact that the inhibitory action depends wholly on the undissociated molecule, HA, and not at all on the other ions present, indicate, in view of the behavior of the other acids examined here, that the action of these acids is not a strictly chemical one. It seems probable that the action of the benzoic and salicylic acid molecules is of the same type as the action of other molecular substances, such as alcohol, or ether. In such a case the cytolytic phenomena observed with higher concentrations, as well as the reversibility under moderate concentrations would be explicable in the same terms.

In conclusion it may be remarked that the anions of these acids possess no sensible toxic properties. Eggs will develop quite normally in relatively strong sea water solutions of sodium benzoate and sodium salicylate, provided the hydrogen-ion concentration is such that the concentration of the free acid is not above the inhibitory threshold. This, of course, is equally true of all the other acids examined here. If a toxic property, similar to that suggested for the molecules of benzoic or salicylic acid, is possessed by the molecules of carbonic, acetic, or propionic acid, it is of too small an order to be perceptible at the concentrations involved in these experiments.

SUMMARY AND CONCLUSIONS

The quantitative relations involved in the action of carbonic, acetic and propionic acid on the process of cell division indicate that the egg cell is freely permeable to both the acid molecules and the anions of these acids. These relations also show that the egg cell possesses a capacity to resist the action of these acids which manifests itself in an apparent retention of a constant quantity of anion in excess of that present in the external fluid. By taking into consideration this departure from otherwise simple equilibrium conditions, it is shown that complete repression of cell division occurs at a constant intracellular hydrogen-ion concentration in solutions containing various concentrations of these acids, and in various mixtures of two of them.

In sea water solutions containing, singly, HCl, lactic acid, the dihydrogen phosphate ion ($H_2PO_4^-$) or the monohydrogen tartrate ion ($HC_4H_4O_6^-$), cell division is repressed only when the hydrogen-ion concentration of the solution exceeds a limiting value (pH 5.0 to 4.6) compatible with cell division. In view of the fact that in the presence of carbonic acid cell division is repressed at an intracellular hydrogen-ion concentration differing not very greatly from pH 6.5, it must be concluded that the egg cell is not freely permeable to lactic acid, $H_2PO_4^-$, $HC_4H_4O_6^-$ or the hydrogen ion.

The failure of sodium lactate, disodium phosphate and disodium tartrate to modify the precise, quantitative action of carbonic or acetic acid on cell division shows that the egg cell is not freely permeable to the lactate ion, the phosphate ion ($PO_4^{=}$) or the tartrate ion ($C_4H_4O_6^{=}$).

In view of the above facts, the assumption is considered untenable that the demonstrated cell-fluid equilibria with respect to the carbonate, acetate and propionate anions is established under any conditions by the actual migration of these anions from fluid to cell; for such an assumption makes necessary a supporting hypothesis of specific permeability on the part of the cell to different anions in order to explain the failure of the other anions examined to migrate under similar conditions. This assumption appears to be irrational and lacking a necessary foundation in chemical theory or experience.

It is inferred that an equilibrium concentration of anion is established in the cell subsequent to, and in consequence of the entrance into the cell of the respective acid molecule, by some mechanism which operates on other components of the cell-fluid system.

The molecules of benzoic and salicylic acids possess "toxic" properties which are apparently not due to their character as acids. In solutions containing these substances, cell division is repressed at constant concentrations of the acid molecule.

I am indebted to my wife for the arduous microscopical work involved in these experiments, which had to be crowded into a short season.

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THE EFFECTS OF CEREBRAL DESTRUCTION ON THE SEXUAL BEHAVIOR OF RABBITS

II. The Frontal and Parietal Regions[1]

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Some thirty-five years ago the attention of clinicians and laboratory workers was focused on the functions of endocrine organs by what is now known as the classical experiment of Brown Séquard (3). This renowned experiment initiated a widespread movement for scientific study of the internal secretions and so great has been its momentum and so general its scope that, in recent years, a great many investigations on problems of sexual behavior have centered directly or indirectly on the endocrine functions of the gonads; temporarily, other aspects of sexual phenomena and their underlying mechanism, particularly the rôle of the central nervous system, have been slighted or neglected. In consequence of this, our knowledge on matters of sex has advanced with an uneven frontage and explanatory principles for sexual phenomena are generally incomplete and tend to over-simplification.

The present study was undertaken for the purpose of determining what portions and what amounts of the cerebral cortex of male rabbits may be removed without causing the animals to lose the reproductive functions.

Pertinent notes from the literature. Gall (5), writing near the first of the nineteenth century, localized the seat of the sexual impulses in the cerebellum. Certain anatomical and clinical observations which had come to his attention caused him to favor this view. He believed that: *a*, cerebellar growth is arrested when animals are castrated at an early age; *b*, the cerebellum undergoes marked atrophy as a result of castration in the adult; *c*, shrinkage of the contralateral hemisphere of the cerebellum follows semicastration; and *d*, general shrinkage of the cerebellum occurs in senility.

In criticism of Gall's claims, it may be said at the outset that they were not substantiated by subsequent anatomical and experimental evidence. The gross weights of the encephala and cerebellums of 10 stallions and 21 geldings were compared by Leuret. Percentage weights of the cerebellums

[1] Financial support for this study was received from The National Research Council through The Committee for Research on Sex Problems.

with respect to the encephala were 11.4 and 13.5 respectively. In this instance the data are actually the reverse of Gall's claims. Hatai's (7) studies of the effects of gonadectomy on the weight of the central nervous system in the albino rat revealed only a slight loss in the gross weight of the brain as a whole following semi- and complete castration. This loss was proportionately distributed to the individual gross divisions of the brain and not confined to the cerebellum. Furthermore, no disproportionate loss of the contralateral hemispheres of the cerebellums following semicastration were reported. With respect to weight changes occurring in senile men, Boyd (2) demonstrated that the changes in gross weights of cerebrum and cerebellum are proportionate for advancing ages from adulthood to senility. In view of the foregoing findings it is obvious that Gall's claims are now deserving of little more than historical reference.

In 1873 Golz (6) reported observations on a partially decerebrate dog from which he concluded that the sexual impulses were regulated by a particular part of the cerebral cortex. This view has been strengthened by extensive researches directed by von Bechterew (1) who described a cortical region in the dog where the application of electrical stimuli gave rise to reflexes of the accessory reproductive organs. Others have been less successful in locating this so-called sexual center in the dog and other animals, yet many writers (Moebius (10), Kraft-Ebbing (8), Loewenfeld (9), Orlowski (12) and others) state or in their writings manifest their belief in its existence.

More recently repeated claims for a sexual center in the cortex which exercises a controlling influence over the reproductive functions of animals have been made by Ceni (4), an Italian investigator. Experiments on cockerels, guinea pigs and dogs, involving traumatic injuries incurred by a blow on the head and surgical lesions, as well, have led him to believe that a center for the control of spermatogenic functions and the regulation of sexual behavior is located in these animals in the dorsal aspects of the cerebral hemispheres. Severe traumatic injury or ablation of this center results in sterility and loss of the "sexual instincts." Upon examining several of the published accounts of Ceni's work it occurred to me that other causes of sterility and impotency might very well have been and probably were operating in the experiments under consideration. To mention a single factor, it seemed probable that nutritional disturbances resulting from traumatic and operative lesions or unbalanced rations actually consumed by the animals studied might have been entirely responsible for the results obtained. For this reason it seemed desirable to repeat the experiments of Ceni with the introduction of such variations in technique as seemed necessary to yield unequivocal data concerning the presence of a cortical center the destruction of which renders the animal impotent and sterile.

Since these experiments were begun another investigator has published a study on the relation of the cortex to the reproductive functions in pigeons which justifies our suspicions as to the inadequacy of the controls in Ceni's experiments. Rogers (13) has shown that the accessible portions of the cortex of pigeons may be removed *in toto* in males and females without sacrificing their ability to beget and rear young. Some of his animals passed successfully through the entire reproductive cycle after deprivation of all the cerebral cortex accessible for ablation. Although one would not be warranted in assuming a priori that these data for the pigeon may be duplicated in the barnyard fowl the relatively close similarity of their brains amply justifies a tentative attitude toward the acceptance of Ceni's findings.

Animals, diet and housing. Two breeds of rabbits have been used in this experiment. They are the Flemish Giants and the Himalaya strains. In a few instances a cross between the two strains, bred in our own laboratory, was employed. No special reason for the selection of these strains of rabbits can be given except the fact that they were available in large numbers in the vicinity of the laboratory and are known to be sturdy animals easily maintained under laboratory conditions.

Since dietary factors are known to be potent agents for modifying, retarding and suppressing the sexual behavior of laboratory animals, special attention was given to the feeding of all subjects before and during the period of experimentation. An ample supply of fresh water and an adequate food ration was provided at all times. The constituent elements of the diet provided were as follows:

Alfalfa hay, ad libitum
Rolled barley, approximately 150 to 300 grams fed each morning and night to a half-grown or adult rabbit
Cabbage and carrots once or twice per week

Whether or not this diet is the best that can be given rabbits we are unable to say, for the present experimental program has not led to an experimental study of this point. In our own laboratory, however, animals fed upon this diet thrive and reproduce throughout the year.

All animals herein considered were reared and maintained in hutches with a floor space for the individual animal or a pair of animals of about three square feet. Some were reared in out-of-door hutches until the age of weaning when they were brought into the laboratory; others were reared and maintained in the laboratory.

Operative technique. The hair was removed from the scalp in order that the skin might be thoroughly saturated with iodine. With animals under deep anesthesia an incision was made in the scalp at a point midway between the eyes. Thence it was carried nasalward one-half inch

and backward approximately one inch. The skin on either side of the incision was retracted and raised from the skull plate. With fine bone forceps an opening in the roof of the skull was made over the region of cortex to be removed. Through this opening a thermo-cautery heated to redness was inserted and a circumscribed portion of the cortex destroyed. Although there was a constant intent to limit the lesion to the cortex, in certain cases, as specified in case histories, the cautery grazed subcortical structures.

After desired portions of the cortex had been cauterized the wound was cleansed with sterile cotton and the edges of the skin brought together and sutured over the opening in the skull. Finally the incision was given several coats of celloidin to seal and protect it during the time required for healing.

Post-operative effects following small lesions were relatively mild. For extensive lesions they were generally severe. During the first week or two subsequent to operations some loss of weight was recorded for all animals and in the younger animals temporary retardation or cessation of growth was observed.

When all necessary data concerning the reproductive functions of operated animals had been collected, they were sacrificed in order that the extent of the cortical lesions might be accurately determined. The brain of each animal was hardened in situ for a few hours and then fixed in Müller's fluid. Eventually each brain was photographed and sectioned. Serial sections (thickness, 40 micra) embracing the full extent of the lesions were stained and mounted. These have been used to determine the depth of the lesion at various points and the amount of injury to subcortical structures. From each series brain sections were selected which illustrate the depth and extent of the lesion at various points. These sections have been drawn under an Edinger projection apparatus at the same degree of magnification and now appear in connection with the individual case histories.

Observations after operation. When this experiment was begun the interest of the experimenter centered chiefly in the immediate effects of cortical destruction on the overt sexual responses of the male. In consequence of this, little attention was given to alterations in spermatogenic functions resulting from these lesions. In later experiments, however, more attention to the latter point has been given. Also, in the early experiments the animals were sacrificed soon after satisfactory evidence of the immediate effects of cortical lesions on sexual behavior had been obtained. In later experiments (to be reported soon) animals were kept alive for several months in order that longer breeding records might be obtained. In the latter cases cortical lesions were more extensive than in the early studies herein reported.

Abbreviations used in description of figures. Aqueductus cerebri, *Aq;* capsula externa, *Ce;* capsula interna, *Ci;* centrum ovale, *Co;* chiasma opticum, *C;* columna fornicis ascendens, *Cfa;* columna fornicis descendens, *Cfd;* columna fornicis horizontales, *Cfh;* commissura fornicis, *Cmf;* commissura anterior, *Cma;* cornu Ammonis, *C.A;* corona radiata, *Cr;* corpus mammillare, *CM;* corpus callosum, *Cc;* fimbria fornicis, *Fi;* fissura Rhinica lateralis, *F R;* ganglion geniculatum laterale, *Ggl;* infundibulum, *Inf;* lamina terminalis, *Lt;* nervus opticus, *II;* nucleus caudatus, *Nc;* nucleus habenulae, *Ha;* septum pellucidum, *Sp;* tractus olfactorius, *Tol;* tractus opticus, *Top;* ventriculus lateralis, *Vl;* ventriculus III, *V III.*

Cross hatching at border of lesions indicates necrotic tissue.

Case histories.[2] An animal in which the cortical lesion is small is used to introduce the series of case histories considered in this paper because in it destruction of the olfactory bulbs and the frontal poles are combined. The case serves to bridge the gap between this and the previous study (14) which is concerned with the destruction of the olfactory bulbs.

Case history 1 (Cross between Flemish Giant buck and Himalaya doe).

Operation: Operated at the age of 2 months when the male had not yet attained sexual maturity. The main objective in this operation was complete separation of the olfactory bulbs with destruction of the most anterior portions of the frontal poles. To insure transection of the olfactory bulbs and destruction of the anterior portions of the frontal poles, a second operation was performed eighty-seven days after the first. At this time the destruction was confined to a small strand of the olfactory bulb on the left side which escaped the cautery in the first operation.

Sexual behavior: Tests of sexual behavior were not made until the eighty-seventh day after the first operation, a few hours before the *second operation.* Upon being confined with a non-receptive female he mounted and *attempted* copulation immediately. No intromission.

One day after the second operation, a receptive female was put into his cage. Copulation and impregnation of the female followed. From this mating a litter of fifteen young was born. The male was approximately 5 months old when the litter was sired. One month after the second operation, age approximately 6 months, he was sacrificed. Smears from the testes and ductus deferentes showed that the reproductive tract was teeming in active spermatozoa.

Extent of brain lesion: The extent of the frontal lesion is shown in figure 1, case 1. On the right the cortex was removed as far posterially as the anterior commissure; the cautery opened the anterior horn of the lateral ventricle and grazed the head of the caudate nucleus on its dorsal surface. On the left, the ventrolateral portion of the frontal pole escaped the cautery and the posterior extent of the lesion is less than on the right side. Neither the anterior horn of the lateral ventricle or the caudate nucleus was touched. On the whole the case presents no points of special interest beyond the fact that transection of the olfactory bulbs and destruction of a relatively small portion of the frontal poles did not noticeably affect the sexual behavior or the reproductive functions of the male.

[2] The terminology used in describing cortical lesions with respect to topographical areas or structures within the brain conforms to that used by Drs. C. Winkler and Ada Potter in their book entitled, *An Anatomical Guide to Experimental Researches on the Rabbit's Brain.* 1911, Amsterdam.

Case history 2 (Flemish giant).

Operation: Operated at the age of 4 months. Destruction began well forward in the frontal regions and extended back into the parietal area. No hyperexcitability followed immediately after the operation or during the first day. On the second day, however, he was very excitable when one approached the cage or attempted to touch him. Gradually excitability subsided and entirely disappeared during the fourth day.

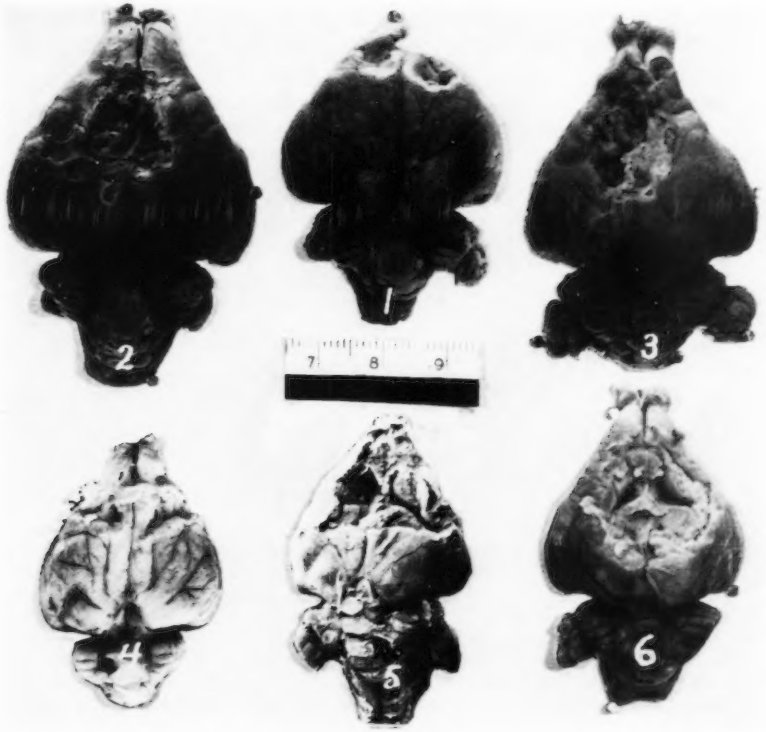

Fig. 1. The photographs of figure 1 illustrate the extent of brain lesions in rabbits described in the text. Nos. *4*, *5* and *6* are approximately normal in size as may be seen by inspecting the centimeter scale which was photographed with them. Nos. *1*, *2* and *3* are slightly magnified. Normally their sizes approximated those of nos. *5* and *6*. The brains were hardened in Müller's fluid.

Sexual record: On the day of the operation this male was put with a receptive female. Two copulations were observed soon after they were put together. He was operated within an hour after sexual behavior was observed.

On the second day after the operation a receptive female was put with him. He mounted two or three times within the first few minutes but eventually ceased copulatory attempts without intromission. On the ninth day four different non-

receptive females were put with him, one at a time. Vigorous attempts to copulate with each of them were made. It seemed probable that had a receptive female been available at this time copulation would have been completed.

Fifteen days after the operation a receptive female was put into his cage. Within a few minutes copulation was observed. Thirty days thereafter the female delivered a litter of six well-developed young. The male was sacrificed on the day he mated with this female.

Extent of the lesion: The photograph of case 2, in figure 1, illustrates the extent of the cortical lesion. (Three niches in the normal cortex of the left hemisphere are to be seen just postero-lateral to the lesion proper. These were made accident-

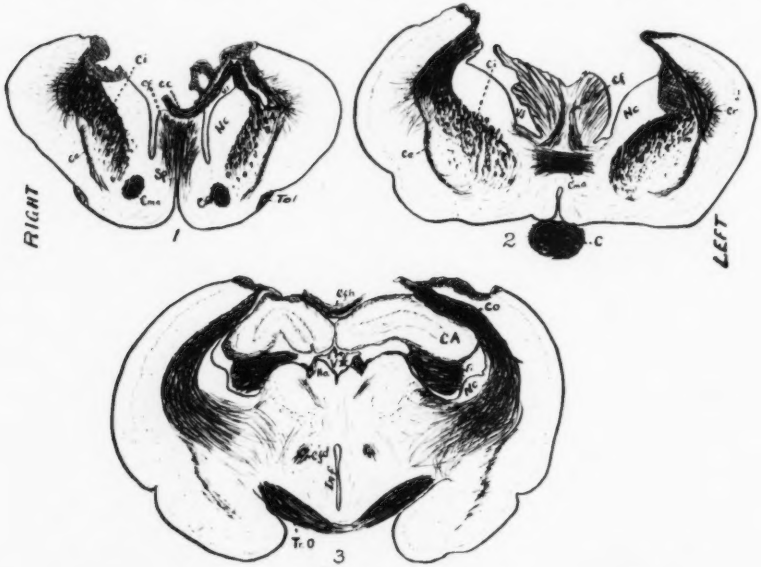

Fig. 2. Sections from the brain of case 2. Section 1 cuts the Telencephalon through the knee of the corpus callosum and orally from the optic chiasma; no. 2, through the optic chiasma, the oral portion of the infundibulum, and the anterior commissure; and no. 3, distally from the optic chiasma and through the proximal part of the thalamus. (Note that the right hemisphere is on the left and the left on the right side.)

ally as the brain was removed from the skull. Otherwise the lesion as it appears in the picture is as made by the cautery.) The lesion includes the greater portion of the precentral and post central areas. It also extends back into the parietal area, especially its medial aspect. No injury to either caudate nucleus is apparent. The following sections, figure 2, reveal the depth and the lateral extent of the lesion at different points in the Telencephalon.

Section 1 cuts the Telencephalon precisely through the genu of the corpus callosum and orally from the optic chiasma. Although the greater portion of the corpus callosum is destroyed a part of it may still be seen on the left side. There it lies in contact with the septum pellucidum and the ascending columns of the

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the left the cornu ammonis connected with the central columns of the fornix by a strong bundle of fibers can be seen; on the right this is entirely missing. The injury to the cornu ammonis extends backward to the distal limit of the lesion on this side. Slight injury to the caudate nucleus may be seen on the left side.

Case history 4 (Flemish Giant).

Operation: Young male approximately 4 months of age when operated. Frontal poles of cortex destroyed. After-effects of operation mild.

Sexual record: Prior to the time of the operation this male had not had an opportunity to attempt sexual congress with a female; he had, however, been observed on several occasions mounting a smaller male with which he was quartered.

Five days after the operation a non-receptive female was put into his cage. He attempted to copulate. Again, 7 days after the operation a non-receptive female was put with him and sexual aggression with repeated attempts at copulation were observed. Twenty days thereafter a receptive female was put with him; within a few minutes he had copulated with this female. The female became pregnant from this mating and delivered a healthy litter. One month after the operation this male was sacrificed. In the ductus deferentes and the testes many active spermatozoa were found.

Fig. 4. Sections from the brain of case 4. No. 1 passes orally to the optic chiasma through the lamina terminalis and the septum pellucidum; no. 2, through the optic chiasma and the anterior commissure.

Brain lesion: The extent of the brain lesion in this male is shown in figure 1, case 4. The lesion embraces most of the area precentralis, the anterior half or two-thirds of the area postcentralis, and extends on either side into the anterior part of the area insularis. The depth of the lesion is shown by the two sections given below in figure 4.

Section 1 cuts the Telencephalon orally from the optic chiasma through the lamina terminalis and the septum pellucidum. The cortex is removed laterally well into the area insularis and all underlying tissue to a depth of and including the corpus callosum. On the left side the head of the caudate nucleus was grazed on its dorso-lateral aspect. The dorsal part of the septum pellucidum was carried away with the corpus callosum and other tissue overlying it.

Section 2 cuts the Telencephalon through the optic chiasma and the anterior commissure. The depth of the lesion becomes less as one goes posterial ward; likewise its lateral extent narrows. In this section the corpus callosum is partially destroyed; but enough remains to give a partial bridge between the hemispheres with its moorings on either side left intact. A portion of the fornix which lies immediately beneath the corpus callosum has been destroyed. The cortex of the area precentralis and about half of the post centralis is gone in this section. No

injury to the caudate nucleus on either side is apparent in this region. The section is near the most posterior limit of the destroyed area.

Case history 5 (Flemish Giant).

Operation: Adult young male approximately 6 months of age. In a single operation the dorsal aspect of the cortex extending posterially into the parietal area was destroyed. Recovery from the operative shock was rapid; animal ate on the following day.

Sexual behavior: From the age of weaning (45 days) this male was kept apart from females. No opportunity for copulation was offered prior to the operation.

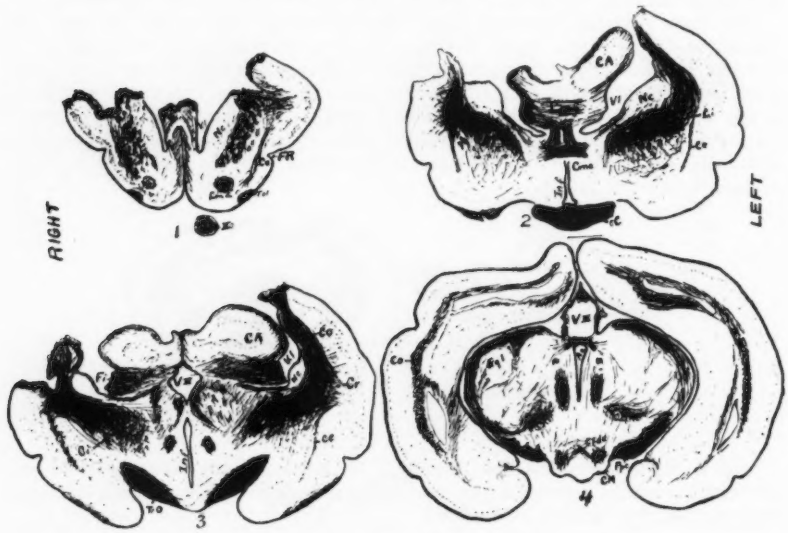

Fig. 5. Sections from the brain of case 5. No. 1 passes anteriorly to the knee of the corpus callosum and through the head of the caudate nucleus; no. 2, through the middle of the optic chiasma; no. 3, through the proximal part of the thalamus; and no. 4 through the posterior commissure dorsally and the corpora mamillare ventrally. (Note that the right hemisphere is on the left and the left on the right side.)

Five days after the operation a non-pregnant female was put into his cage where she was left for 16 days. At the end of the period evidence that she had become pregnant was obtained by palpating the abdomen. Twenty-one days after the operation the male was sacrificed. At this time the testes were large and the scrotum rugose. Active spermatozoa were found in the testes and in the ductus deferentes.

The female impregnated by this male delivered a litter of nine living young on the thirty-third day after being put with the male. *Conception must have taken place on the eighth day subsequent to the operation of the male.*

Brain lesion: The gross extent of the lesion is fully shown in figure 1, case 5. It, together with its border of necrotic tissue, covers, roughly speaking, the areas

precentralis, post centralis, and the anterior half of the area parietalis. On the right, it extends laterally into the superior temporal zone, on the left, where the lateral extent of destruction is less it reaches the border of this zone only. As the picture clearly reveals, the head of the caudate nucleus of the right hemisphere was grazed on its dorsal aspect but not greatly injured. On the whole the lesion affected the Telencephalon chiefly but, as the sections reveal, a part of the Rhinencephalon was touched. The depths of the lesion at various points anterio-posterial ward are shown in figure 5.

Section 1 cuts the Telencephalon just anterior to the genu of the corpus callosum and through the head of the caudate nucleus. The cortex above and lateral to the head of the caudate nucleus excepting a small portion on the left side, was removed; also the corpus callosum and the greater part of the centrum ovale of this region were destroyed. The injury to the caudate nucleus on the right was very slight.

Section 2 cuts the Telencephalon in the middle of the optic chiasma. The corpus callosum and the regions above it were destroyed. On the right the centrum ovale is practically all gone and on the left only a small amount of the most lateral part is left. The injury to the right caudate nucleus is evident in this section. On the right side the cornu ammonis was almost completely removed, but on the left it was barely touched. The columns and commissure of the fornix are visible in this section.

Section 3 is only a short distance posterior to the foregoing section. It cuts the Telencephalon through the proximal end of the thalamus. Centrally and ventrically the third ventricle may be seen. The corpus callosum was destroyed on both sides. On the right all of the centrum ovale is gone and on the left only a small portion is left. The destruction of the cortex ends on the right at the beginning of the superior temporal lobe and in the dorso-lateral convexity of the hemisphere on the left. No injury to the body of the caudate nucleus is apparent on either side. The lateral portions of the fimbria of the fornix were partially destroyed on the right side but untouched on the left.

Section 4 was taken approximately 2 mm. posterior to section 3 and is near the posterior limit of the lesion. It cuts the Telencephalon through the posterior commissure dorsally and the corpora mammillare ventrally. The section is just posterior to the farthest extent of the lesion and shows no destruction of tissue.

Case history 6 (Flemish giant).

Operation: Operated at the age of 4 months. In a single operation the dorsal convexity of the brain was mutilated by cautery. An attempt was made to combine destruction of the areas precentralis, post centralis and parietalis. For three days after the operation the male was very excitable; gradually on the fourth and fifth days hyperexcitability subsided.

Sexual record: This male demonstrated copulatory ability on the day of the operation. Not until the twenty-first day after the operation was a female again put into his cage; no copulatory attempts were made. On the twenty-ninth day he copulated with a receptive female. From this mating a litter of sixteen young was born (fifteen alive and one dead). The litter was sired approximately one month after the operation.

Extent of brain lesion: The extent of the lesion is shown in figure 1, case 6. As this photograph shows, the lesion embraces the greater portions of the areas precentralis, post centralis and parietalis. Anteriorly on the right side the lesion extends into the area insularis; on the left it barely reaches the border of this region. Posteriorly it extends into the proximal portions of the areas retrosplenialis and

occipitalis. By looking closely at the picture one may see that the corpus callosum has been almost completely destroyed throughout its extent; that the caudate nucleus of both sides has been grazed on its dorsal surface; that the commissure and fimbria of the fornix have been partially destroyed; and that the cornu ammonis is mutilated on its dorsal aspect. The extent of the destruction is further illustrated by drawings of sections from various regions within the scope of the lesion (fig. 6).

Section 1 cuts the Telencephalon just distally from the bulbus olfactorius. On either side the Pallium and Rhinencephalon are united dorso-medially. Laterally they are separated by the Fissura Rhinica. The destruction of the Pallium is about equal in amount in the two hemispheres.

Section 2. Judging from visible landmarks this section cuts the Pallium through the knee of the corpus callosum. The ascending columns of the fornix are visible with the anterior cornua of the lateral ventricles showing laterally to them. Just

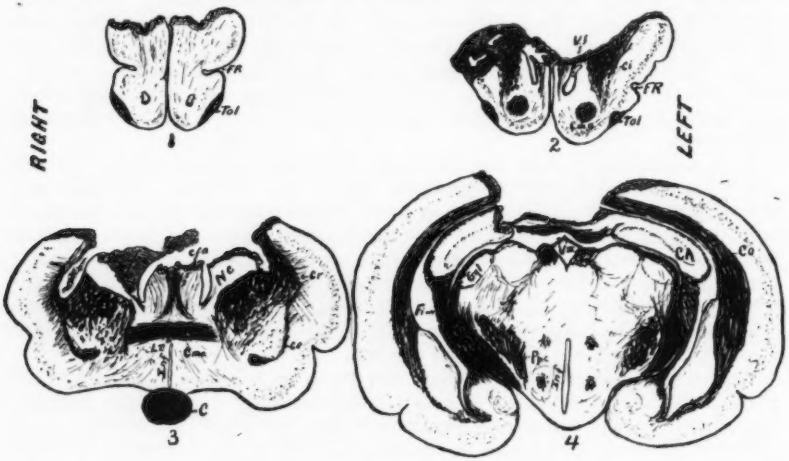

Fig. 6. Sections from the brain of case 6. Section 1 cuts the Telencephalon just distally from the bulbus olfactorius; no. 2, through the genu of the corpus callosum; no. 3, through the oral end of the optic chiasma and the anterior commissure; and no. 4, through the lateral geniculate body and the habenular nucleus. (Note that the right hemisphere is on the left and the left hemisphere on the right.)

outside the ventricles appear the heads of the caudate nuclei. The destruction embraces the whole of the corpus callosum and the tissue overlying it. On the right the cortex is destroyed as far as the Fissura Rhinica and on the left to the dorsal border of the area insularis.

Section 3 cuts the Telencephalon through the oral end of the optic chiasma and the anterior commissure, which unites the two hemispheres at this point. They are already united beneath the anterior commissure by the lamina terminalis. On either side a small portion of the cortex of the area post centralis is left. The whole of the corpus callosum and the dorsal third of the septum pellucidum and columns of the fornix are gone. On both sides the dorso-lateral part of the caudate nucleus is destroyed, and the dorsal portion of the lateral ventricle (cella media) is obliterated as a result of destruction of its roof and portions of its walls.

Section 4. This section cuts the Diencephalon dorsally through the lateral

geniculate body and the habenular nucleus and ventrally through the infundibulum. The section passes through the distal end of the corpus striatum where only the tail of the caudate nucleus is found. Dorsally it cuts the splenium of the corpus callosum in the region where the former structure comes into relation with the commissural fibers of the fornix. On the right the cortical lesion includes the areas retrosplenialis, occipitalis, and part of calcerina; on the left, however, it reaches only the outer border of the area occipitalis. This section is near the posterior limit of the destruction.

SUMMARY

Post-operative behavior of rabbits considered in this study gives no evidence for a "sexual center" in the proximal two-thirds of the dorsal convexity of the cerebral hemispheres. In no case, however, was the extent of the lesion sufficiently great to preclude substitute functioning of the occipital or ventro-lateral portions of the cortex. Neither do the technique employed nor the lesions considered guard against automatic relegation of functions normally mediated by the cortex to subcortical centers following the destruction of these cortical centers. If this took place, however, it is important to note that no special training was necessary to bring it about and that it was accomplished in a relatively short period of time. The data herein presented are inadequate to answer unequivocally the question as to whether a "sexual center" in the dorsal convexity of the cortex actually exists and functions when present. One can only say that cortical destruction in the anterior two-thirds of the dorsal convexity of both hemispheres does not result in the loss of copulatory ability or cause sterility within a relatively short period of time. Further interpretations of these data had best be reserved until case studies in which cortical lesions involving the entire dorsal convexity of the hemispheres have been presented.

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